

QAPP of Land-based, Biological Efficacy

Testing of the OceanDoctor BWMS

Prepared for: Jiujiang Precision Measuring Technology Research Institute

Prepared by: Ballast Water Detecting Lab of Shanghai Ocean University



June 2012

A. Project Management

Quality Assurance Statement

To assure the quality and rationality of the laboratory tests, we hereby declare that the testing for approval and the sampling procedure for ballast water management system conducted by us are strictly in accordance with the requirements of the Quality Assurance Project Plan, the *Guidelines for Approval of Ballast Water Management Systems (G8)* and the *Guidelines for Ballast Water Sampling (G2)*.

Term of validity:

Term of validity of the QAPP; 1th June 2012 to 31th May 2014

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Technical director: Zhang Daiyi Research professor

Quality manager: Sun Anxin Engineer

Jiujiang Precision Measuring Technology Research Institute

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Quality Assurance Statement

To assure the quality and rationality of the laboratory tests, we hereby declare that the sampling and test procedures conducted by us are strictly in accordance with the Quality Assurance Project Plan, the ISO/IEC 17025: 2005, the *Guidelines for Approval of Ballast Water Management Systems (G8)* and the *Guidelines for Ballast Water Sampling (G2)*.

Term of validity:

Term of validity of the QAPP: 1th June 2012 to 31th May 2014

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Note: the quality assurance project supervisor of the test of ballast water management system will keep this file as a project quality assurance record and a basis for the following test.

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A3. Brief Introduction of Participating Organizations

A3.1 Ballast Water Detecting Lab of Shanghai Ocean University

The Ballast Water Detecting Lab of Shanghai Ocean University was founded in September 2008. There are eighteen people in the lab, among which four people are engineers and scientists with high professional title. The lab consists of sample acceptance room, hydrochemistry room, micro organism testing room, microscope room, balance room and sample storage room. The lab is dedicated to the study of the harbor ecology and Invasion ecology, mainly of the ecology research study of the plankton in harbor area and ship ballast water and the micro organisms in ocean environment. This organization has published over 100 papers in both national and international academic journals. In addition, the lab has obtained four patents authorizations.

The lab is equipped with all kinds of instruments and apparatus, such as BOD₅ analyzer, TOC analyzer, spectrophotometer, stereoscopic microscope, conductivity gauge, turbidimeter for water micro-organism test, environmental parameters detection and plankton test. The related staff is asked to be trained before he or she conducts the testing task. The five doctors and thirteen masters are all specialized in the parameter field. By now, the lab is able to test five organism indicators and ten water quality parameters in accordance with the ballast water discharging standards regulated in the International Convention for the Control and Management of the Ships' Ballast Water and Sediments: (1) viable organisms greater than or equal to 50μm in minimum dimension; (2) viable organisms less than 50μm and greater than 10μm in minimum dimension; (3) toxicogenic *Vibrio cholerae* (serotypes O1 and O139); (4) *Escherichia coli*; (5) *Intestinal Enterococci*; (6) heterotrophic bacteria; (7) total residual oxidants (TRO); (8) dissolved oxygen (DO); (9) total suspended solids (TSS); (10) turbidity (NTU); (11) dissolved organic carbon (DOC); (12) particulate organic carbon (POC); (13) pH; (14) salinity; (15) water temperature.

Being realistic and creative, the staff of the lab aims to build a competent and famous lab which is specialized in the testing of ships' ballast water in China.

A3.2 Jiujiang Precision Measuring Technology Research Institute

Jiujiang Precision Measuring Technology Research Institute is a comprehensive research organization which is engaged in precision test, precision processing, and precision measuring. It is a subsidiary company of CSSC (China State Shipbuilding Corporation). It is located in the economic development zone of Jiujiang City. Now, the institute has a total floor area of 92, 800 square meters, building area of 37,000 square meters, and total assets of more than 226 million Yuan (RMB). It also owns more than 1,600 sets of equipments including metering, measuring, processing apparatus and also computers. And it also is equipped with a lot of advanced mechanical and electronic computer aided to design software, analysis software and simulation software.

OceanDoctor BWMS is developed and produced by Jiujiang Precision Measuring Technology Research Institute for the purpose of ballast water treatment. The design and the test of the system are conducted strictly in conformance with the IMO's Guidelines for approval of ballast water management systems (G8), Res. MEPC.174(58) and Procedure for approval of ballast water management systems that make use of active substances (G9) MEPC.169 (57), it is verified by the performance test that the performance specifications are consistent with the D-2 discharge standard stipulated in International Convention for the Control and Management of Ships' Ballast Water and Sediment.

A4. Project Organizational Chart

Research and development organization:

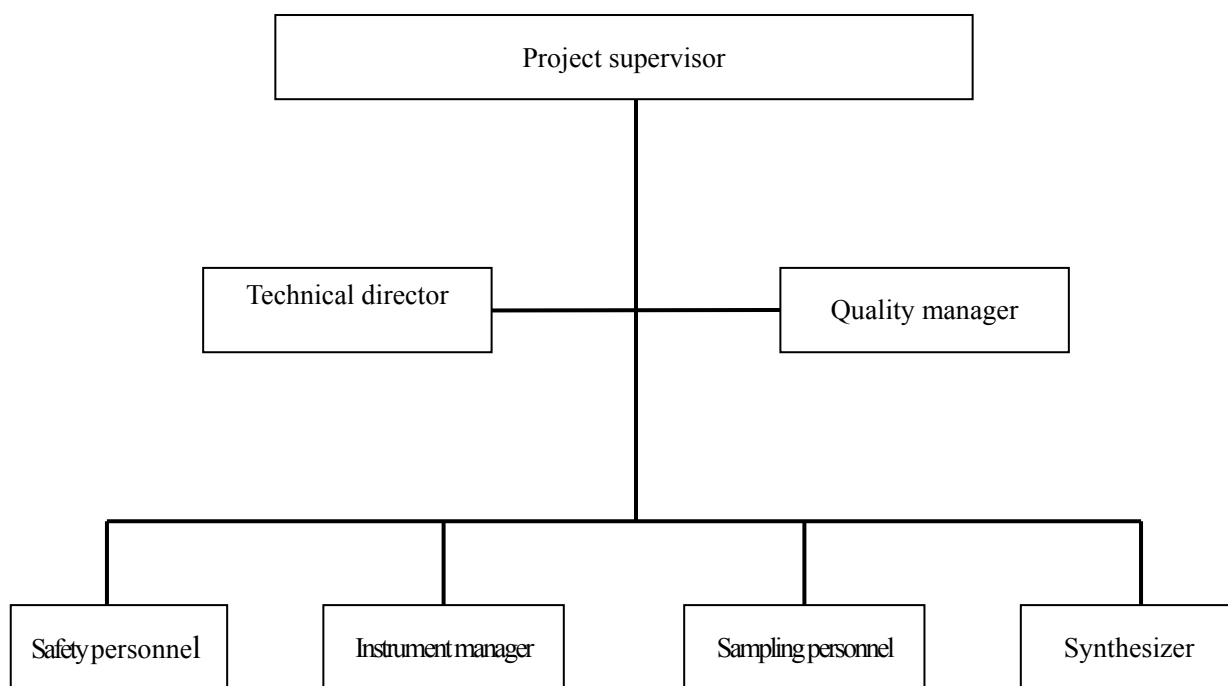


Figure 1 Organizational Chart of the Research and Development Organization

Project supervisor: Li Shulin

The project supervisor is responsible for the overall management work of the project. It is the responsibility of him to organize the human resource; material resource and financial resource of the company to ensure that the project goes on wheels. He is responsible for establishing the quality policy and quality objectives and arranging the work schedule of the project, and also he has the responsibility for urging the staff of the project to follow the requirements of management system files and regulations of the company.

Technical director: Zhang Daiyi

The director is responsible for the overall technical works of the project, and he is responsible for the technical training of the personnel involved in the related test work, arranging the experimental flow and technical principle studying for the related staff. Organizing and coordinating the development of the test is also one of his responsibilities. He is also in charge of dealing with the emergencies occurred during the test process. Moreover, he is responsible for the assurance of test tempo and device status to be in compliance with the requirements of the QAPP.

Quality manager: Sun Anxin

He is responsible for the quality related work and ensures that the quality objectives to be fulfilled. In addition, he is in charge of the safety, healthy and environmental protection work throughout the development of the project. He is responsible for supervising the staff of the project team to finish the work in accordance with the QAPP.

Safety personnel: Liu Gang

He is responsible for the safety of the test field. Keep eye on the safety of the test field, give suggestions on how to deal with the potential safety hazard and monitor the implementation of the improvement measures and ensure the test running in order.

Instrument manager: Ma ji

The operation of the ballast water management system in conformation with the requirements in the test process is one of his responsibilities. And keep record of it. Report to the technical director about the running status of instruments and help the technical director with solving the defaults of the instruments.

Synthesizer: Zhao Jiliang

He is responsible for the management of the document and files over the whole course of the project. Organize and store the documents according to the requirements. He is also in charge of the coordination of the resources in field test.

Sampling personnel: Zhou Yue

He is responsible for assisting the test organizations in collecting the samples .Ensure that the samples are classified and managed in order.

Project test organization:

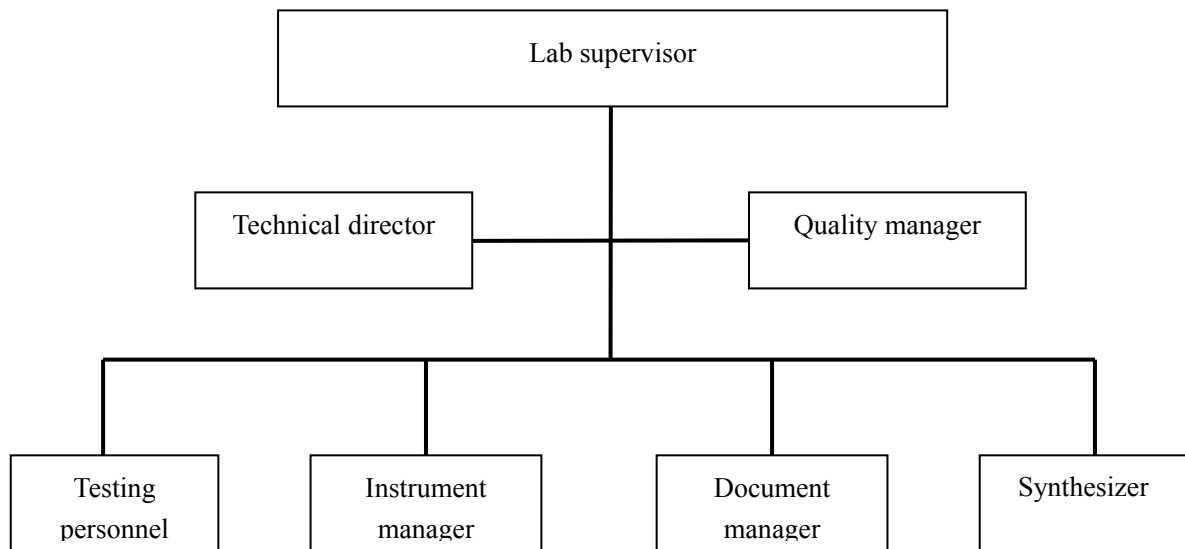


Figure 2 Organizational Chart of the Test Organization

Lab supervisor;Xue Junzeng

He is responsible for the overall project management work and keeping in touch with the research and development organization. He is responsible for the organization structuring and resources allocation. And he is also responsible for establishing the quality policy and quality objectives and arranging the work schedule of the project, and also he has the responsibility of urging the staff of the project to follow the requirements of management system files and regulations of the lab.

Lab director /technical director: Wu Huixian

She is responsible for the overall technical works of the project. Also, she is responsible for the training of the testing personnel and organizing the related staff for test result analyzing; she will supervise and also help the testing personnel to finish the test in accordance with the related requirements and solve the technical problems that may arise in the work process; she is also responsible for making the plan for developing the project and organize the implementation of the plan; moreover, she is asked to ensure the validity of the test standard in use.

Quality manager: Wang Qiong

She is responsible for the quality assurance work for the project and ensures the achievement of the quality objectives. She is also responsible for the custom acceptance

and dealing with the custom complaints. In addition, she is responsible for the safety, healthy and environmental protection work during the project development. And she also plays the role of lab internal affairs supervisor.

Testing personnel: Wang Qiong, Yuan Lin, Bian Jiayin, Liu Liang, Liu Yan, Jiang Jiamei, Yu Jiafeng and so on.

Liu liang and Wang Qiong as the testing personnel of the project are mainly in charge of testing of viable organisms greater than or equal to 10 micrometers and less than 50 micrometers in minimum dimension. Xue Junzeng is responsible for the testing review of the viable organisms greater than or equal to 10 micrometers and less than 50 micrometers in minimum dimension. Wu Huixian and Liu Liang are in charge of the review of the bacteria testing .Yuan Lin is responsible for the testing of the viable organisms greater than or equal to 50 micrometers or more in minimum dimension; Liu Yan is responsible for the testing review of the viable organisms greater than or equal to 50 micrometers or more in minimum dimension. Bian Jiayin is in charge of the testing of the environmental parameters. Liu Liang and Yu Jiafeng are in charge of the review work. In addition, the technical director is responsible for the field sample collection. The sampling personnel are Wang Qiong, Yuan Lin, Wu Huixian, Liu Liang and so on.

The testing staff is asked to conduct the testing work carefully to assure the validity of the testing data. Make sure that the environmental condition of the lab meets the requirements and keep the lab tidy and safety. The staff is required to participate in the training to enhance their awareness of the importance of the quality and the testing ability. The review personnel should master the methods for parameter testing and the uncertainty of the testing result, and review the initial data and testing results objectively and scientifically. All the testing personnel and the review personnel should be responsible for keeping the technical and commercial secrets of the custom.

Field sampling personnel must carry out the sampling work strictly in compliance with the sampling regulations. He or she should fill in the sampling plan and sampling results, and it is field supervisor' responsibility to keep surveillance and fill in the supervision record. Responsibilities of the sampling staff are: the technical director organizes and determines the sampling plan for the organization concerned; the synthesizer

is responsible for the preparation of sampling necessities and the sample acceptance, record-keeping and storage work; the sampling personnel should prepare the sample according to the sampling requirement, and collect the related data and keep records earnestly to ensure the safety and validation of the samples. The leader of the sampling team take charge in the management work during sampling, and he needs to write the work conclusion. Prior to sampling, the technical director should organize the related staff to make a detailed sampling plan according to the testing item and requirement of the entrusted organization, and then verified and approved. The technical director calls sampling personnel together for a meeting to arrange the tasks and explain the sampling requirements, the working contents and the work discipline to them. There should be no less than two experienced staff in each sampling team, and a team leader is appointed to be responsible for the field sampling management. The synthesizer is responsible for the preparation of containers, instrument, sampling list, seal, files, technical standards and letter of introduction needed for sampling. Sampling personnel needs to prepare the stuff and files mentioned above and takes sample according to the sampling plan. The sampling staff should keep record of the data and operations relating to sampling which is an integral part of the testing. Sampling record should include the sampling procedure, the identification of sampling personnel, and the site map of the sampling locations as appropriate. There should be no less than two personnel taking part in field sampling. The record kept in field should be clear, detailed, integrated, and the sampling personnel and the representative from the entrusted organization should sign on the sampling list together. The sampling personnel seal the sample in situ according to related regulation, and signature of the representative from the entrusted organization may be needed as necessary. Once the samples are sealed, no one is permitted to change or make a replacement. And the sampling personnel should strictly follow the work principles to ensure the authenticity, unbiased and representativeness of the sample.

Instrument manager: Yuan Lin

He is responsible for the maintenance of the instruments. Keep operation record and maintenance record of the instrument. He also takes charge in the calibration of the instrument and preparation and custody of the instrument record card.

Document manager: Liu Liang

The document manager is responsible for the classification, cataloging and custody of the documents. He is responsible for the filing and managing of test reports and documents related. One of his other responsibilities is to file and manage the technical documents such as standards, regulations, procedures and system documents and the personnel technical documents as well. Moreover, he is asked to keep the file room safe and clean and make sure that the documents in good conditions. He is also the sample keeper and responsible for the classification and record of the sample. He is responsible for keeping the environment of the sample room in normal condition and he should make the sample room safe and clean and also keep the samples in good condition. He is responsible for the distribution of the test reports in time.

Synthesizer: Yuan Lin

He is responsible for preserving the testing samples in right conditions taking the requirements of the custom into consideration. He is responsible for storage and management of the consumables. He is also responsible for supervising and inspecting the storage and dispose situations of dangerous goods. And he is responsible for acceptance of the custom's testing samples and appendices and keeping record of the status characteristics of those; responsible for the test work for the external custom, sample number and status identification; store the samples required to be held in time and keep the availability and integrity of the sample in the storage period. He is responsible for the custom service, getting access to the requirements of the custom and satisfying their needs. In addition, he takes the responsibility for delivering the feedback information to the person related in order to improve the quality management system. He is in charge of dealing with the complaints and he is asked to summarize the requirements of the custom and report to the quality manager in time. He is responsible for the preparation of the facilities and environmental conditions for test. He is responsible for compiling test reports, and then the test reports are stamped and delivered by his.

The lab manager should ensure that the staff is qualified for performing the specialized equipment operation, testing, result assessment, test report sign and certificate verification. If anyone who has not completed training is to be assigned to finish one task,

he or she will be supervised according to the Supervising work Control Procedures. For those who undertake specialized work, there should be qualification confirmation corresponding to their education, training, experience, specific test requirements and certifiable skills. The specific requirements are as follows: for those people who are color blindness should not undertake the tests concerning color identification. For those people who undertake the biotic experiments should know well about the knowledge of bio-test safety operation and sterilization. The chemical parameter authorized signatory should have the undergraduate degree or above in chemical, and moreover, he or she should have the technical working experience for three years at least. If not, he or she should have worked in the chemical related field for at least 10 years.

A5. Project Background

With the rapid development of the world trade and global tourism, the demand for freedom trade is growing and the marine shipping industry is exuberant and occupies 60% share of world trade. To assure the safety of sailing ships, it is necessary to add some ballast to keep the ship in an appropriate stable and floating status. Since 1980s, it is common to use water as ballast, and it is the so called ballast water. While the ballast water makes it easier for the spread of species from one water region to another one. Once ballast water in ship containing harmful aquatic organisms or pathogens is discharged to the waters of another port state, it will endanger the local ecology, economy and human health, and the effect will last for a long time. Once the aquatic organisms invade and inhibit in the local waters, they will reproduce in an uncontrollable manner, then destroy the food web of local species. And these disastrous causes will lead to mass propagation of harmful parasite and pathogen and even extinguish the local species.

The test and management of ballast water is getting more and more important as the ocean pollution is getting worse and worse due to the discharging of the ship ballast water. Aiming to prevent the potentially devastating effects of the spread of harmful aquatic organisms and pathogens carried by ships' ballast water from one region to another. IMO proposed and approves the International Convention for the Control and Management of

the Ship's Ballast Water and Sediments. Regulations stipulate that: (1) the average density of organisms greater than or equal to 50 micrometers in minimum diameter in the replicate samples is less than 10 viable organisms per cubic metre; (2) the average density of organisms less than 50 micrometers and greater than or equal to 10 micrometers in minimum diameter in the replicate samples is less than 10 viable organisms per millilitre; (3) the average density of *Vibrio cholerae* (serotypes O1 and O139) is less than 1 cfu per 100 milliliters, or less than 1 cfu per 1 gramme (wet weight) zooplankton samples; (4) the average density of *E. coli* in the replicate samples is less than 250 cfu per 100 milliliters; (5) the average density of intestinal *Enterococci* in the replicate samples is less than 100 cfu per 100 milliliters.

A6. Project/Task Description

A6.1 Description of OceanDoctor Ballast Water Management System

A6.1.1 System components and functions

OceanDoctor BWMS is mainly composed of a filtration unit, a photo-catalytic reaction unit, a control unit and the sampling facility.

In order to reduce space occupation, all units can be installed independently. They can either be installed on a common base which is fit for new building ships or installed separately in conformity with the real installation space of the ships which is fit for existing ships. The general arrangement drawing of the system is as shown in figure 3.

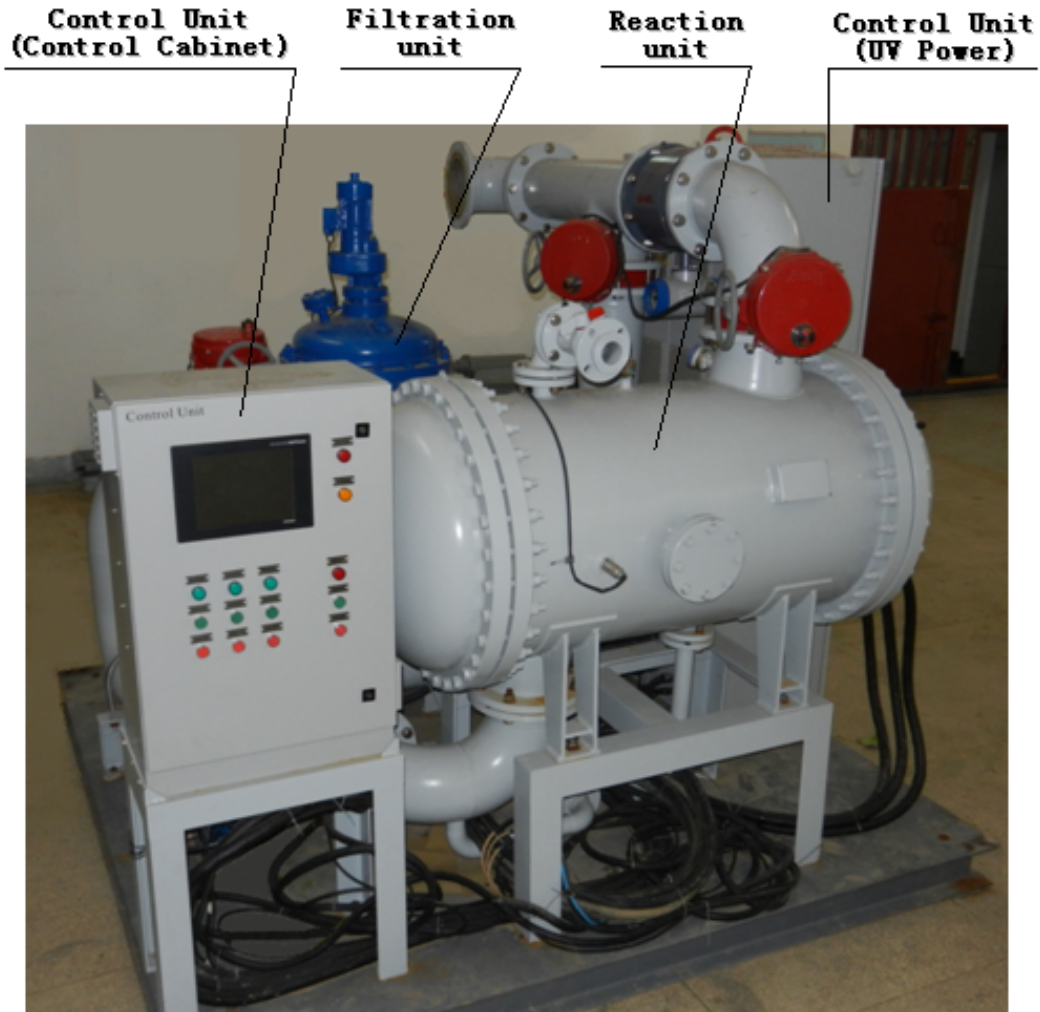


Figure 3 OceanDoctor BWMS

A6.1.1.1 Filtration Unit

A filter with auto backflushing function is selected to prevent the particles and organisms greater than or equal to 50 μm in minimum dimension from passing the filter to the ballast tank and eliminate the accumulation of sediments in ballast tank.

The procedure of the filter includes two independent parts: filtration and auto backflushing. When the filter is backflushing, the filtering process is not suspended, so the backflushing has no effects on the treatment efficiency.

A6.1.1.2 Photo-catalytic Reaction Unit

The photo-catalytic reaction unit is mainly composed of the reaction chamber, UV lamp assembly, photo-catalytic reaction film and supersonic cleaner.

The UV light irradiated by low-pressure mercury lamps inside of the reaction unit can disinfect the organisms in ballast water as the ballast water passes through the photo-catalytic reaction unit. The UV irradiates on the surface of photo-catalytic reaction film, initiating a chemical reaction, generating hydroxyl radicals. The hydroxyl radical is a kind of powerful oxidant and it can disinfect organisms like algae and bacteria in ballast water in quite a short time.

A6.1.1.3 Control Unit

The control unit is mainly composed of electric cabinet, control cabinet and monitor sensors.

The control unit provides power supply for the proper operation of the ballast water management system. And it has the functions of auto-control, status monitoring, displaying, recording, storage and alarming for the system ballasting, deballasting and emergency bypassing. The control unit is also equipped with a printer interface to realize printing function by connecting a printer.

A6.1.1.4 Sampling Facility

Two sampling facilities are installed in the system: sampling facility 1 which is installed in the inlet of the ballast water management system (prior to filtration unit), and sampling facility 2 which is installed in the outlet of the photo-catalytic reaction unit. The influent water samples and the discharged water samples are taken from sampling facility 1; and the treated ballast water samples are taken from sampling facility 2.

The design of the sampling facility conforms to the requirements stipulated in G2.

A6.1.2 Working Procedure

A6.1.2.1 Ballasting

When ballasting, the seawater is pumped by the ballasting pump and After the organisms and sediments greater than or equal to 50 μ m in minimum dimension are filtered in the filtration unit, the seawater is then treated in the photo-catalytic reaction unit to

disinfect organisms in ballast water. The treated ballast water enters the ballast tank through pipes..

During ballasting, by setting the differential pressure of the filter or by time presetting. The auto cleaning of the filtration unit could be controlled; when the ballasting is over, the supersonic cleaner is activated automatically, starting washing and cleaning the sleeves of the UV lamps.

A6.1.2.2 Deballasting

When deballasting, the filtration unit and the photo-catalytic reaction unit are out of service. The water in ballasting tank is pumped out and discharged to the outboard directly through pipes.

A6.2 Test Base and Test Set-up

A6.2.1 test base

The test will be conducted in the Ballast Water Detecting Laboratory of Shanghai Ocean University Land-Based Testing Base. This test base is located at the No.2 dock of Yang shan deep water harbor in Shanghai.

The ballast water test set up is built in the test base; the photo of the test base is shown in the figure 4:

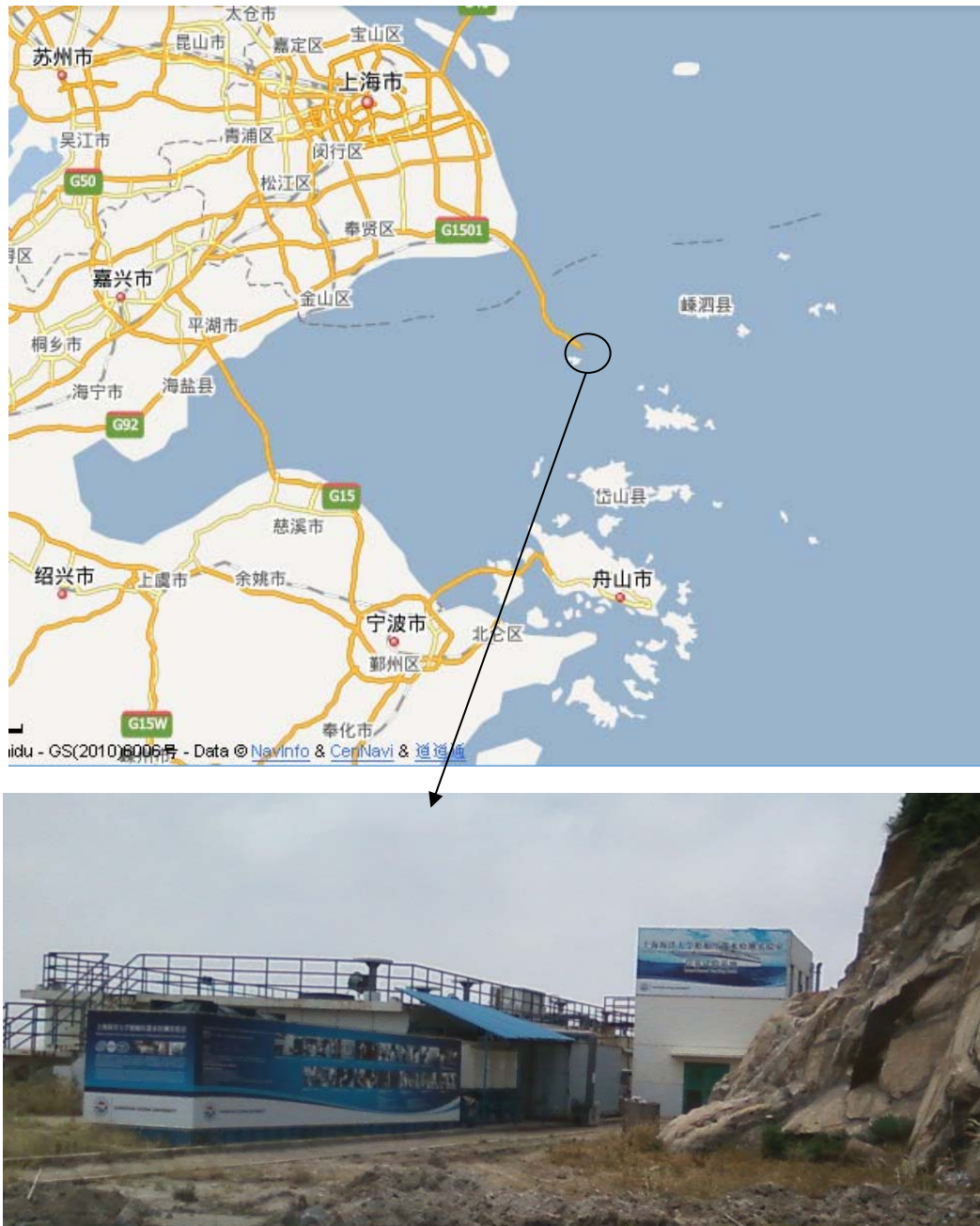


Figure 4 Location and Photo of the Test Base

A6.2.2 Test Set-up

The test set-up is mainly composed of the OceanDoctor BWMS with a treatment capacity of $250 \text{ m}^3/\text{h}$, a ballast pump, a treated tank, a control tank, water feed tank, fresh water tank, sampling facility and so on. This test set-up can realize the function of water ballasting, storage and discharge to meet the approval test requirements. The diagrammatical drawing of the test set-up is shown in figure 5:

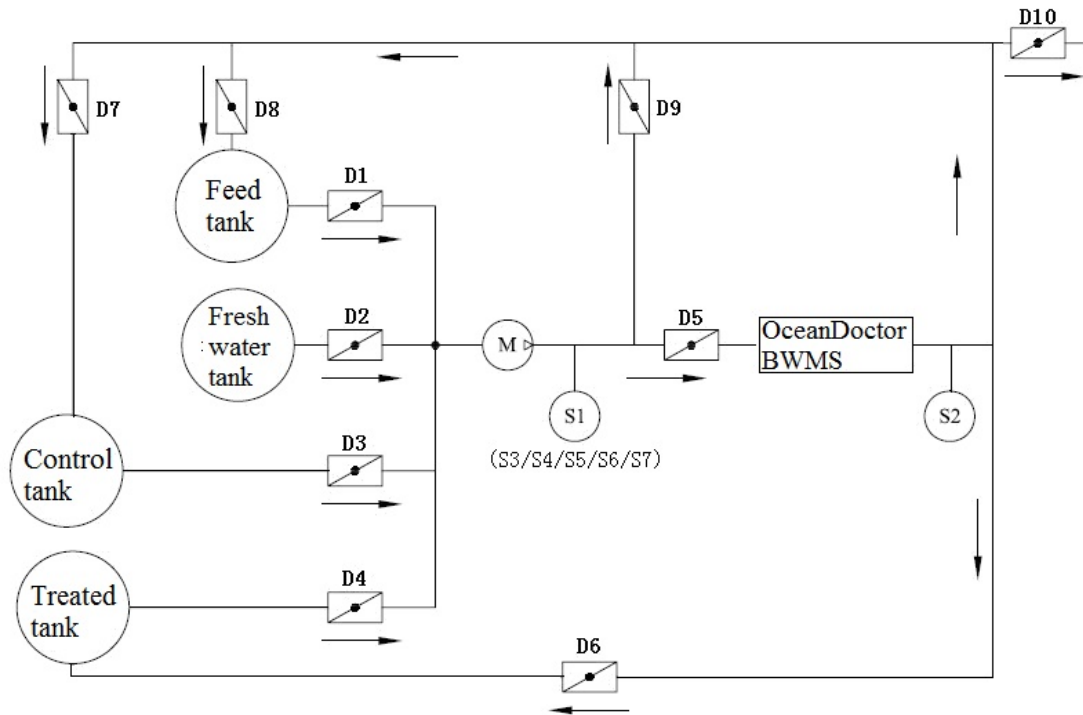


Figure 5 Diagrammatical Drawing of the Test Set-up

OceanDoctor BWMS: it is mainly encompassed by the filtration unit, the photo-catalytic reaction unit, the control unit. The system is applied to disinfect the aquatic organisms like algae and bacteria in ballast water with a TRC of 250 m³/h.

Ballast pump: it will be utilized to pump the water to the ballast water management system. The capacity of ballast pump is 300 m³/h.

Treated tank: it will be utilized to store the treated ballast water with a volume of 250m³. The inside wall of the tank is covered with marine cabin paints.

Control tank: it will be used to store the untreated ballast water with a volume of 250m³. The inside wall of the tank is covered with marine cabin paints.

Feed tank: an aeration device is installed inside of it. This tank is utilized as a container for intake water augmentation to achieve challenge conditions. The volume of the feed tank is 500 m³.

Fresh water tank: it will be used for fresh water storage to wash the test system and pipe prior to or after test. The volume of it is 50m³.

Sampling facility: there are two sampling points in the system and seven sampling ports (S3, S4, S5, S6, S7 is the same port with S1, which are utilized to take different

samples), S1 is used for intake water sampling; S2 is used for treated water sampling; S3 is utilized for control water sampling taking from the control tank; S4 is used to take treated water samples after holding for 24h; S5 used to take treated water samples after holding for 120h; S6 is used to take control water samples after holding for 120h; S7 is used to take control water samples after a 24h hold.

The design of the sampling facility is in consistent with the G2 guideline. The structural design of the sampling facility is shown in figure11:

Piping: direct the seawater in the pipe by adjusting the valve.

A6.2.3 Tested equipment

Parameters of the OceanDoctor BWMS for land-based testing are as bellow:

- Treatment capacity;250m³/h
- Power supply voltage;220V
- Grade of filtration;50μm



Figure 6 OceanDoctor BWMS to be tested

A6.2.4 test water

A6.2.4.1 regulations on test water

The challenge water might be classified in to the raw water and the augmented water.

- The raw water refers to the natural seawater with no artificial additives, and it

sometimes may fulfill the influent water criteria as required in G8;

- The test water refers to the challenge water which is purposely amended by adding certain biological or chemical composites to the influent water to assure the test water meet the challenge water conditions in the IMO G8, the target organisms should include common local species or the common used test species.

Seawater water quality parameters in the Yang shan deep water harbor, refer to table1 for detailed information:

Table 1a Water Parameters of the Yang shan Deep Water Harbor

Parameter	Unit	Yangshan deep water harbor	Influent water requirements in G8
Total Suspended Solids (TSS)	mg/L	595.3	>50
salinity	psu	15.0~19.0	3-32
zooplankton($\geq 50\mu\text{m}$)	cell/m ³	2.8×10^3 (12 species from 5 different phyla/divisions)	>100,000 (at least 5 species from at least 3 different phyla/divisions)
phytoplankton(10 – 50 μm)	cell/mL	121(8 species from 1 phyla/division)	>1,000(at least 5 species from at least 3 different phyla/divisions)
Heterotrophic bacteria	cfu/mL	2500	>10,000

Water parameters in east sea area of Shengshan island of Zhoushan city:

As listed in table 1b;

Table 1b Water parameters in east sea area of Shengshan island of Zhoushan city

Parameter	Unit	East sea area of Shengshan island	Influent water requirements in G8
Total Suspended Solids (TSS)	mg/L	25.3	>1
Salinity	psu	32.2~33.0	>32
zooplankton ($\geq 50\mu\text{m}$)	cell/m ³	1.78×10^4 (9 species from 6 different phyla/divisions)	>100,000 (at least 5 species from at least 3 different phyla/divisions)
zooplankton (10 – 50 μm)	cell/mL	15.8(10 species from 2 different phyla/divisions)	>1,000(at least 5 species from at least 3 different phyla/divisions)
Heterotrophic bacteria	cfu/mL	5.5×10^4	>10,000

Chemical composition requirement;

According to G8, for any given set of test cycles (5 replicates is considered a set) a salinity range should be chosen. At least two sets of tests cycles should be conducted, each with a different salinity range and associated dissolved and particulate content Tests under adjacent salinity ranges in the above table should be separated by at least 10 PSU. For the given salinity, the dissolved and particulate content that should have in test water is shown in table 2:

Table 2 Test Water Quality Requirement

	Salinity (PSU)	DOC (mg/L)	POC (mg/L)	TSS (mg/L)
High salinity test cycle	> 32	> 1	> 1	> 1
Medium salinity test cycle	3 - 32	> 5	> 5	> 50

Biological composition requirement;

If cultured organisms are needed, *Escherichia coli*, rotifer, artemia nauplii and

microalgae might be chosen to be used. The raw water or the augmented water utilized for biological test should meet the following criteria in table 3:

Table 3 Requirement on the Concentration of Test Organisms in Test Water

	Organism concentration
$\geq 50 \mu\text{m}$	$10^6 \text{ cell/m}^3 - 10^5 \text{ cell/m}^3$, (at least 5 species from at least 3 different phyla/divisions)
10-50 μm	$10^4 \text{ cell/ml} - 10^3 \text{ cell/ml}$, (at least 5 species from at least 3 different phyla/divisions)
Viable heterotrophic bacteria	10^4 cell/ml

A6.2.4.2 preparation of the test water

Meet the chemical requirement:

Medium salinity test cycle:

Seawater in the Yangshan deep water harbor is pumped and settled in a sedimentation tank, suspended solids in natural seawater bigger in size are settled, and then the water is pumped to the feed tank. Organisms like zooplankton, algae, and bacteria are added to assure the physical/chemical and biological characteristics of the influent water (for land-based test) meet the criteria stipulated in G8. The test base is situated in the East sea area, the normal salinity of the natural seawater is about 17.0~21.0 PSU, so it can be utilized as the medium salinity test water.

High salinity test cycle: natural seawater is pumped at the east sea area of Sheng shan island of Zhoushan city (S5 in figure 7) which is 50 nautical miles from the test base and shipped to the test base, and then pumped to the feed tank. Organisms like zooplankton, algae, and bacteria are added to assure the physical/chemical and biological characteristics of the influent water meet the criteria stipulated in G8. Because the salinity of the natural seawater is about 32.2~33.0 PSU, it can be utilized as the high salinity test water directly.



Figure 7 Map of the Location of High Salinity Test Water

Meet the organism concentration requirement:

The marine organisms added to the test water are cultured by Shanghai Ocean University as required by the test. During the test, zooplankton (rotifer, protozoa,artemia) ,algae (dinoflagellate, green alga, diatom and so on) and heterotrophic bacteria(*Escherichia coli*)in specific concentration are amended to the feed tank, the water in the tank is aerated by the aerating apparatus to keep the organism alive and also well mixed and distributed in the water. Seawater and microorganisms utilized for testing purpose are pumped by the ballast pump to the piping. By this way, the organism concentrations in the influent water could meet the requirement in G8.



Figure 8 Organism Culture

A6.2.5 Test cycles

Based on the test objectives, test cycles are classified into the biological efficacy test cycle for assessing the environmental acceptance and the test cycle for verification of biological efficacy (land-based test). This QAPP applies to the test cycles for verification of biological efficacy (land-based test).

A6.2.5.1 test cycles for biological efficacy testing (land-based test)

The test cycles should be conducted as follows:

- The ballast water is pumped and treated in line.
- The treated water and the untreated control water are held for 5 days in the simulated ballast tank and the control tank, respectively.
- The ballast water is discharged by the pump;

A6.2.5.2 measures taken for preventing cross-contamination

Prior to each test or between test cycles, the treatment tank and the control tank should be washed by the high pressure fresh water, and after that, clean them with a rag to get rid of the foreign substances like the fragments, organisms and so on.

The cleaning of the treatment tank and the control tank should be performed as follows:

- .1 Prior to each test, fill the involved tanks with freshwater and also rinse the pipes;
- .2 high pressure freshwater is injected to the wall of the tank from top to the bottom;
- .3 drain the remained water in the bottom of the tank by a submersible pump.
- .4 wipe the tank with a clean rag again until it is thoroughly clean(visual inspection)

.5 Dry the treatment tank and the control tank with a drying blower.

To prevent cross-contamination between test cycles, all the sampling equipment, such as the sampling barrel, the sampling bottle, the sieve should all be washed by freshwater.

A6.3 Test Schedule

The test schedule is made immediately after the assignment of the entrustment agreement. And the test procedures will be strictly following the test schedule.

Refer to table 4 for detail:

Table 4 Project Schedule

date item	June 2012	July	August	September	October
QAPP	√				
Test preparation	√	√	√		
Organism culturing		√	√	√	√
System commissioning		√			
Test cycle			√	√	√
Data determination			√	√	√
Data analysis			√	√	√
Test report					√
Conclusion					√

The determination of the testing parameters is in accordance with the test contents and the schedule of the project. See details in project parameters table (table 5)

Table 5 Project Test Parameters

No.	Test item	No	Test item
1	pH	9	Dissolved Organic Carbon (DOC)
2	turbidity (NTU)	10	10-50 μm viable organisms
3	salinity (PSU)	11	≥ 50 μm viable organisms
4	temperature (T)	12	Heterophic bacteria
5	Dissolved Oxygen (DO)	13	<i>Escherichia coli</i>
6	Particulate Organic Carbon (POC)	14	<i>Intestinal enterococci</i>
7	Total Residual Oxidants (TRO)	15	<i>Vibrio cholerae</i> (serotypes O1 and O139)
8	Total Suspended Solids (TSS)		

A7. Objectives of Testing Result

The objectives of the test data are to ensure the objectiveness and scientificity of water quality parameters (environmental parameters) and organisms' parameters. Error data falling outside of the controlled range is invalid. Organism parameters before treated by OceanDoctor BWMS are: test organisms with minimum dimension greater than or equal to 50 μm which consist of at least 5 species from at least 3 different phyla/divisions in certain concentrations should be no less than 10^5 per cubic meter; overall density of the tested organisms with minimum dimension less than 50 μm and greater than 10 μm which consist of at least 3 different phyla/divisions in certain concentrations should be no less than 10^3 per cubic meter; concentration of viable heterophic bacteria should be less than 10^4 cell/ml. The treated water after being held for 120h is to be in accordance with the

regulation stipulated in the International Convention for the Control and Management of the Ships' Ballast Water and Sediments: less than 10 viable organisms per cubic meter greater than or equal to 50 µm in minimum dimension; less than 10 viable organisms per milliliter less than 50 µm in minimum dimension and greater than or equal to 10 µm in minimum dimension; Toxicogenic *Vibrio cholerae* (O1 and O139) with less than 1 (cfu) per 100 milliliters; *Escherichia coli* less than 250 cfu per 100 millilitres; *Intestinal enterococci* less than 100 cfu per 100 milliliters. Viable organism concentration in discharged water from the control tank untreated by OceanDoctor BWMS meets the requirements that exceeding 10 times of the treated water.

The salinity dissolved organic carbon (DOC), particulate organic carbon (POC), total suspended solids (TSS) of the tested water should meet standards as required.

A8. Test Training Assurance

A8.1 Sample Collection and Handling

The sampling and sample preparation personnel should be trained in accordance with the Guidelines for Ballast Water Sampling G2 and the Specification for Marine Monitoring GB17378.4-2007. The way to determine the needs for training and person to be trained should meet the requirements of the Personnel Training Procedures of Ballast Water Detecting Lab of Shanghai Ocean University. The training plan should be made with reference to the project tasks currently and expected. The availability of the training is to be evaluated. During the training, the trainer should get to know every step of the sample collecting and preparing. The trainer should be supervised by the experienced staff in the lab to conduct the sample collecting and preparing work.

A8.2 Lab Test and Analysis

The test and analysis staff of the lab should be trained according to the Personnel Training Procedures of Ballast Water Detecting Lab of Shanghai Ocean University; Specification for Marine Monitoring GB17378.4-2008, GB17378.7-2008; Water quality. Determination of free chlorine and total chlorine: Spectrophotometric Method Using

N,N-diethyl-1,4-phenylenediamine HJ 586-2010; Observation Method for Gulf Ecosystem, Standard Examination Methods for Drinking Water—Indicator Microbes GB/T 5750.12-2006; Diagnostic Criteria for Cholera WS 289-2008, Water quality - Detection and Enumeration of intestinal enterococci - Part 2: Membrane Filtration Method (ISO 7899-2:2000); Guidelines for Approval of Ballast Water Management Systems G8; Procedure for approval of ballast water management systems that make use of active substances G9. Those workers who will undertake the chemical related work in the lab should learn how to protect and rescue themselves. The important chemical test staff (those who knows well of the test methods, procedures, objectives and result assessment) should master the assessment method for determination of uncertainty of environmental parameters analysis. The organism test staff should know the safe handling and sterilization procedures of organism test. All the staff should be assured to be qualified and supervised to carry out the work according to the management system.

The lab manager should ensure that the staff is qualified for performing the specialized equipment operation, testing, result assessment, test report sign and certificate verification.

A9. Documents and Records

A9.1 QAPP

The development and research organization and the test organization will discuss and determine the QAPP prior to the implementation of the project. And the QAPP will be handled and recorded as the project controlled document.

A9.2 Field Running Record

To make sure that the system operate within the normal parameter range in the test cycle, running status of the ballast water management system is recorded.

Table 6 Running Record Table of the Ballast Water Management System.

No;

entrustment organization	Jiujiang Precision Measuring Technology Research Institute		
Test equipment	Model HBS-250 OceanDoctor ballast water management system		
Test location	Yang shan deep water harbor Pudong new area of Shanghai Land based test base of Ballast Water Detecting Lab of Shanghai Ocean University		
Test cycles		Test date	
Test phase	ballasting <input type="checkbox"/> holding <input type="checkbox"/> deballasting <input type="checkbox"/>		
Start time		End time	
Supervision unit	Wuhan Branch of China Classification Society	Supervisor	
Test related information	Equipment Running status		
	Running parameter	Flow; Dose;	
	sampling related information	Biological efficacy water sample <input type="checkbox"/> Relevant chemicals water sample <input type="checkbox"/> Biological toxicity water sample <input type="checkbox"/>	

recorder;

quality manager;

project supervisor;

A9.3 Field Sampling Record

Information about the collected sampling data will be recorded on the water proof table and the information in table is about the date, sampling personnel, weather, environmental condition, sample lot number and the identified sample quantity. Any deviations from the standard sample procedure or emergencies should all be recorded.

The label of related sample (figure 9) ;

Sample ID:	
Sampling Time:	Sampling temperature:
Sampling humidity:	Sampling point:
Sampling personnel:	Supervisor:

Figure 9 Sample Label

The samples collected will be named according to standard identification serial numbers;

The sample ID code: SHOU-BWDL-JPMT –tri-digit serial number +abbreviation of the test classification +the order of the sampling parallel. The abbreviation of the test items are as follows: A represents viable organism sample with a dimension of 10~50 μ m; B represents viable organism sample greater than 50 μ m; C represents micro organism sample; D represents environmental parameter sample; for example: SHOU-BWDL-JPMT-001A1 represents the first replicate sample among the first lot samples with a dimension of 10~50 μ m viable organisms delivered by Jiujiang Precision Measuring Technology Research Institute and tested by ballast water detecting lab of Shanghai Ocean University.

Table of sampling record (table 7) ;

Table 7 Record of Sampling Result

No; _____

Sample name			
Sampling date		Sampling location	
No. of sampling point		Sampling time of 1# replicate sample	
Sampling time of 2#replicate sample		Sampling time of 3#replicate sample	
No. of samples		Environmental factors	Temperature: °C Humidity: %RH
Sampling results	Organisms greater than or equal to 50 micrometers or more in minimum dimension:		
	Organisms greater than or equal to 10 micrometers and less than 50 micrometers in minimum dimension:		
	Bacteria:		
	Environmental parameters		

A9.4 Record of the Chain of Custody

To assure the quality control of the project, keep record of the chain of custody. Table of record of chain of custody (table 8):

Table 8 Record of Chain of Custody

No; _____

Test task number		Custody date	
Test item		Custody personnel	
Test objective			
Test personnel			
Custody contents			
Contract (letter of authorization)	Complete <input type="checkbox"/> Lack of contents <input type="checkbox"/> No <input type="checkbox"/>		
Test plan	Complete <input type="checkbox"/> To be <input type="checkbox"/> No <input type="checkbox"/>		
Instrument	Calibration status qualify <input type="checkbox"/> Allow to use <input type="checkbox"/> Forbidden to use <input type="checkbox"/>		
Personnel	Qualification Hold qualification card <input type="checkbox"/> No qualification card <input type="checkbox"/>		
Sample	Quantity		
	Appearance quality Qualified <input type="checkbox"/> Unqualified <input type="checkbox"/>		
	Unique mark Have <input type="checkbox"/> No <input type="checkbox"/>		
	Sample preparation record Have <input type="checkbox"/> No <input type="checkbox"/>		
Test method	Right <input type="checkbox"/> To be completed <input type="checkbox"/> Problem <input type="checkbox"/>		
	Standard (regulation) Have <input type="checkbox"/> No <input type="checkbox"/>		
Environment	Temperature °C	Humidity %RH	
	Condition: Qualified <input type="checkbox"/> Unqualified <input type="checkbox"/>		
Record	Original record: meet the requirement <input type="checkbox"/> Do not meet the requirement <input type="checkbox"/> no record <input type="checkbox"/>		
	Operation record: meet the requirement <input type="checkbox"/> Do not meet the requirement <input type="checkbox"/> no record <input type="checkbox"/>		
Response action of custody			
Remarks			

A9.5 Laboratory Original Data Records

The original data in the lab must be recorded clearly, and the records will be stored in an appropriate facility to keep them away from damage or losing, and also be accessed easily. Preservation period of the record must be specified and all the records should be kept secret. The collection, retrieval, access, file, storage, maintenance and cleaning of the quality record and technical record should be in compliance with the Control Procedures of Ballast Water Detecting Lab of Shanghai Ocean University.

The lab should preserve the detailed records of the information about original observation, educe of data, verification route, calibration records, personnel record and the copies of report distributed within the stipulated preservation period. Each test record or calibration record should include the following information: sample number, test date, standards, test conditions and so on. This could help to identify the factors of uncertainties and assure the repeatability of the test or calibration in conditions similar to the original. The content of the records should involve information about the name of the sampling personnel, the test personnel, verification personnel. If culture medium is prepared, it is necessary to make a record of the name and type of the culture medium; marks of the preparation time and preparation personnel; culture medium/solution type and volume; volume of the sub-package; composition, content, manufacturer and lot number of each composition,; pH value (initial and final); complementing means, time and temperature of sterilizing measures and etc should be recorded.. Keep record of the observation result, data and calculations in situ, and. Identify the records according to the requirements of specified tasks.

If the lab records need to be modified, two lines should be written on the original records, and don't erase the original records. Then the modified records should be written near the original records with the mender's stamper or signature or abbreviative signature.

Table of original record of the phytoplankton test (table 9) ;

Table 9 Original Record of the Viable Organisms 10~50μm in Minimum Dimension (seawater)

Project title			Project No.		
Date of entrustment			Test date		
Sample ID code			Sample		
Applicable standards	GB17378.7-2007 sedimentation method; phytoplankton (seawater) SOP				
Test conditions	temperature; °C, humidity; %RH				
instrument	Phytoplankton counting box, optical microscope (), slide, pipettor				
Sample acceptance time			Concentrated volume mL(V ₁)		
Sampling volume L(V)			Start and end time of the test	~	
Test result					
First replicate Measurement	Testing volume(V ₂);	Second replicate measurement	Testing volume(V ₂);	Third replicate measurement	Testing volume(V ₂);
species name (Latin name)		species name (Latin name)		species name (Latin name)	
Total count (cell) X		Total counting (cell) X		Total counting (cell) X	
density (cell/ml) D ₁		density (cell/ml) D ₂		density (cell/ml) D ₃	
Average density (cell/ml) D ¹					
Note; density (D) =X*V ₁ /(1000V*V ₂) average density (D ¹) =(D ₁ +D ₂ +D ₃)/3					

Test personnel;

reviewed by;

date;

date;

Table of original record of the zooplankton test (table 10) ;

Table 10 Original Record of the Viable Organisms $\geq 50\mu\text{m}$ in Minimum Dimension

Project title			Project No.	
Entrustment			Test date	
sample ID			Sample description	
Applicable	GB17378.7-2007, part 7			
Test conditions	temperature; $^{\circ}\text{C}$, humidity; %RH			
instrument	optical microscope () , stereo microscope ()			
Sample receiving time		Start time		
Volume of filtered water (m^3)		End time		
Testing result				
<div>Species name</div> <div>quantity</div>				
Total count (cell)				
Total density (cell/ m^3)				
Note; total density (cell/ m^3) = total count (cell) / volume of filtered water (m^3)				

Testing personnel;
date;

reviewed by;
date;

Table of original record of the bacteria testing (table 11a, table11b, table11c) ;

Table 11a Original Record of the Total Plate Count and the *Escherichia coli* Testing

Sample name;

Date received; date of testing;

Test location and ambient condition: microorganism detection room temperature;
°C relative humidity; %RH

Test instrument; 100ml cylinder, clean bench, 0.1ml and 1ml
Pipette, timer, incubator, spreading rod.

Test methods; GB/T 5750.12. 1, 2.1, 2.2, 4.1, 4.2-2006; GB 17378.7-2007.10.1, 9.1; *Microbial Test Instruction*

Testing results and records;

Testing results and records;

- (1) Total plate count (freshwater) ;pour nutrient agar into each plate of the decimal scale potency dilution samples, and a plate without sample
- (2) Total plate count (seawater) ;take 0.1ml of bacteria sample at each dilution gradient to spread it to the 2216 plate, add blank, then incubated the plate at 36°C ,then examined after 7 days. If sample concentration is relatively low, pipette 1 mL of water sample and place it to the plate.
- (3)total coliforms (Multiple tube fermentation method) ;five double lactose peptones (each with a volume of 10ml) are incubated at 37°C for 24h. If any acid or bubble is generated, vaccinate the Eosin methylene blue plate, and make it incubated at 37°C for 24h, choose the suspicious colony for Gram staining and inoculate the Lactose peptone at the same time, and make it incubated at 37°C for 24h. Referment to generate acid and bubble. Gram-negative no bacillus gives a positive result in coliform group test. If it is gram-negative budless bacillus, then we say the result is bacteria positive. (4) *Escherichia coli* (Multiple tube fermentation method) ;transform the positive tube in to EC-MUG, observe that under the UV light, if there is blue fluorescent, it indicates positive result. Inoculate the positive tube as mentioned in(2) to EC-MUG at 44.5°C for 24h, observe

under the UV lamp, The presence of bright blue fluorescence is considered a positive response for *E. coli*.

(5) total coliform (membrane filtration method) ;100ml of water sample is filtered(in the condition that the concentration of the water sample is relatively high, dilute the water sample by two gradient) on the germ free membrane, place the membrane on the Fuchsin sodium Sulfite culture medium at 37°C for 24h. choose the suspicious colony for gram staining, and inoculate the Lactose peptone at the same time at 37°C for 24h. referment for observation of acid or bubble, Gram-negative no bacillus gives a positive result in coliform group test. If it is gram-negative budless bacillus, then we say the result is bacteria positive.

(6) *Escherichia coli* (membrane filtration method) ;inoculate the positive membrane to the NA-MUG, incubated at 37°C for 4h, observe under the UV lamp, The presence of bright blue fluorescence is considered a positive result.

Sample ID code	total plate count (cell/mL)						Total coliform (MPN/100mL) (CFU/100mL)		<i>Escherichia coli</i> (MPN/100mL) (CFU/100mL)	
	undiluted	10 ⁻¹	10 ⁻²	10 ⁻³	10 ⁻⁴	result		result		result
SHOU-BWDL-										
SHOU-BWDL-										
SHOU-BWDL-										
SHOU-BWDL-										
SHOU-BWDL-										
SHOU-BWDL-										
SHOU-BWDL-										
SHOU-BWDL-										
SHOU-BWDL-										

blank control;

positive control;

Total plate counting;

Total coliform;

test personnel;

reviewed by;

date;

date;

Table 11b Original Record of *Vibrio cholerae* Testing

Sample name;

Date received; date of testing;

Test location and ambient condition: microorganism detection room temperature;

°C relative humidity; %RH

Test instrument; cylinder, clean bench, Pipette, timer, incubator at 37°C.

Test methods; WS 289-2008 appendix A

Testing results and records;

A volume of 450ml Water sample is collected,

add 0.5ml of phenothalin(1%) and 0.3ml of potassium tellurate. Adjust the pH to 8.4 -9.2 with 1 mol/L of sodium hydroxide solution.

Add 50mL of ten times of APW, after cultured in the incubator for 6h at 37°C, inoculate to the TCBS plate at 37°C, and then examined after 24h, visually observe if there is growth of suspicious colony.

Sample ID;SHOU-BWDL-	yes(),no()whether there is growth of suspicious colony
sample ID;SHOU-BWDL-	yes(),no()whether there is growth of suspicious colony
sample ID;SHOU-BWDL-	yes(),no()whether there is growth of suspicious colony
sample ID;SHOU-BWDL-	yes(),no()whether there is growth of suspicious colony
sample ID;SHOU-BWDL-	yes(),no()whether there is growth of suspicious colony
sample ID;SHOU-BWDL-	yes(),no()whether there is growth of suspicious colony
sample ID;SHOU-BWDL-	yes(),no()whether there is growth of suspicious colony
sample ID;SHOU-BWDL-	yes(),no()whether there is growth of suspicious colony

test personnel;

reviewed by;

date;

date;

Table 11c Original Record of Intestinal enterococci Testing

Sample name;

Date received; date of testing;

Test location and ambient condition: microorganism detection room temperature;

°C relative humidity; %RH

Test instrument; cylinder, clean bench, Pipette, timer, incubator (37°C, 44°C)

Test methods: ISO 7899-2: 2000

Testing results and records:

100ml of water sample is filtered by a germ free filter(dilution operation might be conducted when the concentration of water sample is relatively high), place the filter membrane on the Intestinal enterococci culture medium by membrane filtration method, the plate is culture at 37 for 44h. Observe if there is growth of colonies in red, nut brown or pink.

If there is any typical colony, transfer the filter membrane to the bile esculin azide agar plate which has been preheated to 44°C .under this condition the colony is cultured for 2h at 44°C . Then observe the plate, if the color of the culture medium around the colonies is brownish black, it means the colonies are positive, these colonies is counted as *Intestinal enterococci*.

sample ID;SHOU-BWDL- yes () ,no () whether there is growth of suspicious colony; total count of positive colony

sample ID;SHOU-BWDL- yes () ,no () whether there is growth of suspicious colony; total count of positive colony_____

sample ID;SHOU-BWDL- yes () ,no () whether there is growth of suspicious colony; total count of positive colony_____

sample ID;SHOU-BWDL- yes () ,no () whether there is growth of suspicious colony; total count of positive colony_____

sample ID;SHOU-BWDL- yes () ,no () whether there is growth of suspicious colony; total count of positive colony_____

sample ID;SHOU-BWDL- yes () ,no () whether there is growth of suspicious colony; total count of positive colony_____

sample ID;SHOU-BWDL- yes (),no () whether there is growth of suspicious colony; total count of positive colony_____

sample ID;SHOU-BWDL- yes (),no () whether there is growth of suspicious colony; total count of positive colony_____

sample ID;SHOU-BWDL- yes (),no () whether there is growth of suspicious colony; total count of positive colony

test personnel;
date;

reviewed by;
date;

(table 12) ;original record of environmental parameters testing

Table 12 Original Record of Environmental Parameters Testing

Project No.;

No;_____

Title of the Project				Test parameter									
Entrustment date				Sampling date									
Sampling description													
Standards		.											
Test conditions		Temperature: °C Humidity:		Test method									
Experiment preparation													
Instrument													
Instrument model													
Test date													
Test time													
Test results													
No .	Water depth	Sampling point	Sample ID code	Determination value				In situ Water temperature $t_w/^\circ\text{C}$	Calibration				in situ pH_w
				1	2	3	Aver age						
Remarks													
Calculation method													
Test reports number													
Tested by									Reviewed				

B. Project test and Data Acquisition

B1. Test Preparation

B1.1 Test Condition Preparation

The technical director will take charge in dividing the staff into the sampling group and the test group and assigning task for each team. She is responsible for preparing the related regulations, standards and operating instructions according to the testing task. To determine the instruments, apparatus and the environmental conditions, and purchase and check the chemical reagents and consumables needed for experiment is also one of her responsibilities. Preparation of such stuff as the blank records, sampling bottles, sample labels, sampling facilities, and sample handling reagents and so on is one of her responsibilities as well.

Preparation of the environmental conditions: Pressure-washed the feed tank, treated tank, the control tank and all the pipes with tap water, dried and swept before starting testing procedures to remove loose debris, organisms and other matters. Check the water source, power supply to be in good condition. Finish the tank intake preparation one day before experiment, and the water static and aeration preparation. Check the technical parameters of the treatment device and get ready for the performance test of treatment.

Testing organisms of greater than or equal to 50 μm in minimum dimension and test organisms greater than or equal to 10 μm and less than 50 μm in minimum dimension which consist of at least 5 species from at least 3 different phyla/divisions in certain concentrations will be collected and cultured, and also *Escherichia coli* in certain concentration and volume will be cultured as stipulated in the guidelines for approval of the *Guidelines for Approval of Ballast Water Management Systems (G8)*. Finish preparing the required concentration of organisms before the testing cycle started; the test will be performed until the organisms in the feed tank are well mixed.

B1.2 Test Items and Test Methods

The lab will conduct the organisms testing and the tests for nine environmental parameters, heterotrophic bacterium, *Escherichia coli*, *Intestinal enterococci*, Toxicogenic *Vibrio cholerae* (serotypes O1 and O139) and organisms with two kinds of ranges for particle size according to the guidelines for approval of the *Guidelines for Approval of Ballast Water Management Systems (G8)*, and the requirements of the project entrustment organization. Refer to table 13 for the detailed testing items and methods.

Refer to table 14 for the calibration requirements of the instruments that will be used in the test.

Table 13 Testing Items and Methods

Testing Target	No.	Testing Items	Testing Methods
Microbes	01	<i>Escherichia coli</i>	The specification for marine monitoring Part 7:Ecological survey for offshore pollution and biological monitoring GB17378.7-2007/9.1,9.2 Standard examination methods for drinking water - Microbiological parameters GB/T 5750.12-2006/4.1 , 4.2
	02	<i>Vibrio cholerae</i>	Diagnostic criteria for cholera WS 289-2008
	03	<i>Intestinal enterococci</i>	Water quality Detection and enumeration of <i>Intestinal enterococci</i> Part 2: Membrane filtration method BS EN ISO 7899-2:2000
	04	Heterotrophic bacteria	The specification for marine monitoring Part 7:Ecological survey for offshore pollution and biological monitoring GB17378.7-2007/10.1
Phytoplankton	05	Phytoplankton (10-50μm)	The specification for marine monitoring—Part 7:Ecological survey for offshore pollution and biological monitoring GB17378.7-2007/5.3.2.3
zooplankton	06	zooplankton (≥50μm)	The specification for marine monitoring—Part 7:Ecological survey for offshore pollution and biological monitoring GB17378.7-2007/5.3.3.3
Environmental parameters	07	Total Suspended Solids (TSS)	The specification for marine monitoring—Part 4:Seawater analysis

Testing Target	No.	Testing Items	Testing Methods
			GB17378.4-2007/27
	08	Dissolved Organic Carbon DOC(mg/L)	The specification for marine monitoring—Part 4:Seawater analysis GB17378.4-2007/34.1
	09	pH	The specification for marine monitoring—Part 4:Seawater analysis GB17378.4-2007/26
	10	Dissolved Oxygen(DO)	The specification for marine monitoring—Part 4:Seawater analysis GB17378.4-2007/31
	11	Temperature (T)	The specification for marine monitoring—Part 4:Seawater analysis GB17378.4-2007/25.1
	12	Turbidity (NTU)	The specification for marine monitoring—Part 4:Seawater analysis GB17378.4-2007/30.1
	13	TRO	Water quality.Determination of free chlorine and total chlorine.Spectrophotometric method using N,N-diethyl-1,4-phenylenediamine HJ 586-2010
	14	Particulate Organic Carbon POC(mg/L)	Gulf ecosystem observation method China Environmental Science Press 2005 / 4.5.14.1
	15	Salinity (S)	The specification for marine monitoring—Part 4:Seawater analysis GB17378.4-2007/29.1

Table 14 Calibration Requirements of the Testing Instruments

No	Parameters Tested	Equipment Name	Model	Measuring Range	J expand uncertainty/k largest tolerance/1 level accuracy	Frequency of Verification Calibration Requirement
01	Heterotrophic bacteria	GZX-III serial light incubator	GZX-400BS-III	(0~60)°C	$U=0.3^{\circ}\text{C}, (k=2)$ $\pm 0.2^{\circ}\text{C}$	annually
02	<i>Escherichia coli</i>	GZX-III serial light incubator	GZX-400BS-III	(0~60)°C	$U=0.3^{\circ}\text{C}, (k=2)$ $\pm 0.2^{\circ}\text{C}$	annually
03	<i>Vibrio cholerae</i> (serotypes O1 and O139)	GZX-III serial light incubator	GZX-400BS-III	(0~60)°C	$U=0.3^{\circ}\text{C}, (k=2)$ $\pm 0.2^{\circ}\text{C}$	annually
04	<i>Intestinal enterococci</i>	LDZX model vertical pressure steam sterilizing pot	LDZX-75KBS	(50~126)°C	$U=0.5^{\circ}\text{C}, (k=2)$ $\pm 0.2^{\circ}\text{C}$	annually
		DK model electric-heated constant temperature water bath kettle	DK-S26	(RT+5~99)°C	$U=0.3^{\circ}\text{C}, (k=2)$ $\pm 0.3^{\circ}\text{C}$	annually
05	Phytoplankton (10-50μm)	biological microscope	S8APO	(40~1600)X	$\pm 5\%$	annually
06	Zooplankton (≥50μm)	biological microscope	DM500	(40~1600)X	$\pm 5\%$	annually
		stereomicroscope	PXS	(10~80)X	$\pm 5\%$	annually

No	Parameters Tested	Equipment Name	Model	Measuring Range	J expand uncertainty/k largest tolerance/1 level accuracy	Frequency of Verification Calibration Requirement
		Plankton net	50 μm	≥50 μm	A grade	annually
07	Total Suspended Solids	electronic balance	AL104/01	(0.0001~110)g	I grade	annually
08	Dissolved Organic Carbon	TOC determinator	TOC-V CPH/CPN	(0.01~1000)ug/L	±0.5mg/LC	annually
09	pH	pH meter	FE20 model	(0~14)pH	0.01grade	annually
10	Dissolved Oxygen	side piston automatic zero setting burette	25mL(Ex)	(0~25)mL	B grade	annually
11	Temperature	thermometer	(0~40)°C	(0~40)°C/0.2 °C	0.62 °C	annually
12	Turbidity	WGZ-3turbidimeter	WGZ-3	(0~1000)NTU	3.90%	annually
13	Total Residual Oxidants	ultra violet spectrophotometer	UV-2000 model	(190~2600)nm	IV grade	annually
14	Particulate Organic	ultra violet spectrophotometer	UV-2000model	(190~2600)nm	IV grade	annually

No	Parameters Tested	Equipment Name	Model	Measuring Range	J expand uncertainty/k largest tolerance/1 level accuracy	Frequency of Verification Calibration Requirement
	Carbon					
15	Salinity	salimeter	SYA2-2	2~42	$U=0.0038(k=2)$	annually

B2. Test and Sampling Design

B2.1 Testing Process

B2.1.1 function testing

To comply with the requirement of the Testing Program, make sure that all the components of the BWMS runs in normal, and all the electrical control functions well.

B2.1.2 performance test

Land-based test cycles will be conducted in accordance with G8 for the performance test. The procedure of the test cycles in the land-based test are given as follows:

1) Challenge water augmentation

When carrying out the medium test cycle, raw water is pumped to the sedimentation tank, after being settled for 12h; the water is pumped to the feed tank. Then, to assure the physical/chemical and biological characteristics of the influent water (for land-based test) meet the criteria stipulated in G8, the raw water is amended with zooplankton, algae, bacteria and so forth.

When carrying out the high salinity test cycle, high salinity test water is taken from east sea area of Sheng shan island of Zhoushan city (S5 in figure 7) which is 50 nautical miles from the test base and then shipped to the test base and pumped to the feed tank. To assure the physical/chemical and biological characteristics of the influent water (for

land-based test) meet the criteria stipulated in G8, the raw water is amended with zooplankton, algae, bacteria and so forth.

2) Processing in treated tank

The testing water in the feed tank will be pumped to the testing system, the test water flow to the filter, and then it will be directed to the photo-catalytic reaction unit, finally, the treated water will flow to the treatment tank and be kept in a tightly closed condition.

3) Processing in control tank

The test water in the feed tank is pumped to the pipes; the test water will flow directly to the control tank through the bypassing pipe, and then stored air-tightly.

4) Storage

The treated water will be stored for 5 days in the treated tank and the control tank, in respectively. Keep the tanks off from light. Rinsing and drying the feed tank and the pipes in this time.

5) Treatment tank deballasting

The treated water in ballast tank will be pumped by the ballast pump into the pipe and discharged directly after holding for 120h.

6) control tank deballasting

After a 120h hold, the water in the control tank will be pumped by the ballast pump to the pipes and treated by the BWMS, then discharged to the sea.

7) To prepare for the next testing, the treated tank, the control tank and the pipes should be rinsed and dried after one test cycle is over.

B2.2 Sampling Facilities and Sampling points

B2.2.1 sampling points arrangement

The test water in the feed tank will be pumped to the pipes, water sample S1 is collected in the upstream pipe before water flows to the treatment unit, and water sample S2 is taken in the pipe post the treatment unit; water sample S3 is taken before the water flows to the control tank. The test cycle is finished when the 250 m³ treatment tank and the 250 m³ control tank are filled and then the both tanks are closed. After a five day hold in

ballast tank and control tank, take the sample S5 in the discharge pipe of the ballast tank. For the effluent water from the control tank; collect the sample S6 before water is treated by the OceanDoctor BWMS. The sampling facilities are designed for compliance with G2. For the isokinetic sampling, a quantitative water sampler is applied to collect the sample. For collecting the bacteria sample, using the sterilized sampling bottle by way of underwater sealing sampling. The arrangement of the sampling points is as shown in figure 10.

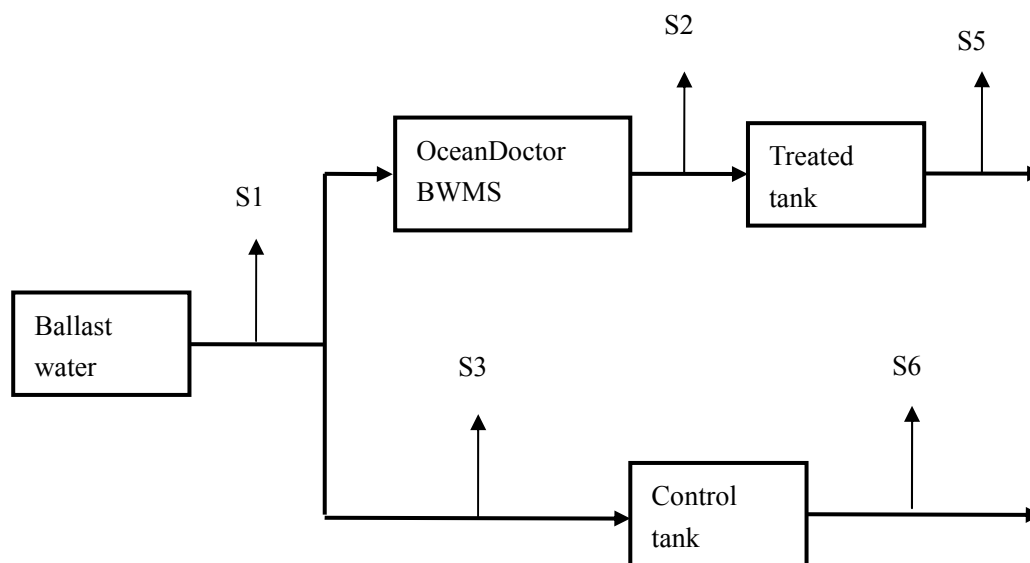


Figure 10 Diagrammatic Drawing of Sampling Points

S1: influent water;

S2: treated water;

S3: control water

S5: treated tank discharge after a 120h hold

S6: control water after a 120h hold

B2.2.2 sampling facility

According to the regulations in IMO convention and G2 guidelines, the sampling facility should be positioned such that a representative sample of ballast water is taken. The sample should be representative of the main stream. The characteristic of the test equipment is taken into account in deciding where the sampling point should be

appropriately positioned.

The design of sampling point adopts the isokinetic diameter calculation in accordance with G2, the associated regulations in G2 mainly includes:

① Through computation fluid dynamics modeling, it has been shown that the isokinetic diameter calculation can provide guidance for sizing determination of sampling points for sampling of organisms.

② Simulations showed that flow transitions from the main stream were best for sampling point diameters between 1.5 and 2.0 times the isokinetic diameter. Ports sized in this range had smooth transitions and pressure profiles that allowed for direct sampling without the need of a pump to induce sample collection. The isokinetic sampling point diameter should therefore be determined generally according to the equation:

$$Diso = Dm\sqrt{Qiso / Qm}$$

Where *Diso* and *Dm* are the diameters of the sampling point opening and the main flow in the discharge line, respectively; and *Qiso* and *Qm* represent the respective volumetric flow rates through the two pipes. It is recommended that sampling point size be based on the combination of maximum sample flow rate and minimum ballast flow rate that yields the largest isokinetic diameter.

③ The opening of the sampling pipe should be chamfered to provide a smooth and gradual transition between the inside and outside pipe diameters.

④ The length of the straight sample pipe facing into the flow can vary, but should not usually be less than one diameter of the sampling pipe. The sampling port should be oriented such that the opening is facing upstream and its lead length is parallel to the direction of flow and concentric to the discharge pipe which may require sampling pipes to be “L” shaped with an upstream facing leg if installed along a straight section of discharge pipe.

In the ballasting, the total volume of test water $V=500\text{m}^3$, the sampling size: $VI=4\text{ m}^3$, in the deballasting, the discharge water volume after treated $V=250\text{ m}^3$, the sampling size; 4 m^3 , the sample port diameter is calculated based on the sampling rate in deballasting.

The treatment capacity of the system $Qm=250\text{m}^3/\text{h}$, so the test duration is:

$$T = \frac{V}{Q_m} = \frac{250\text{m}^3}{250\text{m}^3/\text{h}} = 1\text{h}$$

When the sampling size V_1 equals to 4 m^3 , the volumetric flow rate is:

$$Q_{iso} = \frac{V_1}{T} = 4\text{m}^3/\text{h}$$

The diameters of the main pipe $D_m = 200\text{mm}$, so

$$D_{iso} = D_m \sqrt{Q_{iso} / Q_m} = 200 \sqrt{4 / 250} = 25.3\text{mm}$$

Flow transitions from the main stream were best for sample port diameters between 1.5 and 2.0 times the isokinetic diameter, so the sample diameter range are;

$$(1.5 \sim 2.0) * 25.3 = (37.95 \sim 50.6)\text{mm}$$

Therefore, the internal diameter of the sampling point is designed to be 40mm.

The sample points are distributed as shown in figure 11:

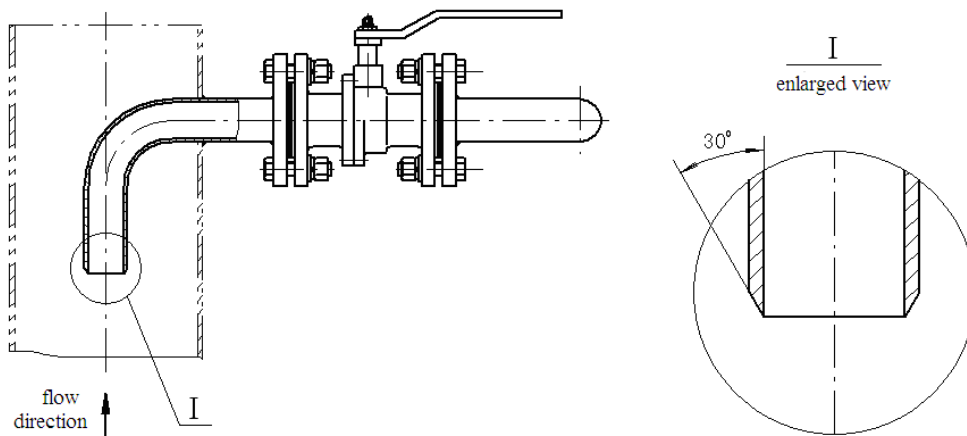


Figure 11 Drawings of the Sampling Facility

B2.2.3 sampling protocol

The sampling protocol should result in samples that are representative and in random with appropriate sample size, and the sampling method should be in line with these G2 Guidelines. Samples for the following three categories should be collected respectively:

- .1 Organisms greater than or equal to $50\mu\text{m}$ in minimum dimension;
- .2 Organisms greater than or equal to $10\mu\text{m}$ and less than $50\mu\text{m}$ in minimum dimension;

.3 *Escherichia coli*, Intestinal enterococci, vibrio cholerae (serotypes O1 and O139) and heterotrophic bacteria.

B2.2.4 sampling size

To determine the water quality indicators, organisms and bacteria, sample three times at each sampling point in different time period, the sampling size and the test item are shown in the table 15.

Table 15 Sampling Size and Number of Sample Taken from Each Sampling Point

No.	Sampling target	Sampling point	Sampling size			
			$\geq 50\mu\text{m}$	10~50 μm	bacteria	Water quality
1	Influent water	S1	20L*3	1L*3	0.7L*3	5L*3
2	0h treated water	S2	1m ³ *3	10L*3	0.7L*3	5L*3
3	0h control water	S3	20L*3	1L*3	0.7L*3	5L*3
4	Discharge water after a 120h hold	S5	1m ³ *3	10L*3	0.7L*3	5L*3
5	Control water after a 120h hold	S6	1m ³ *3	10L*3	0.7L*3	5L*3

B2.3 Sampling Phase Design

Samples are collected at a sequence of beginning, middle, and end. In cases when the treatment capacity is 250m³/h, the total time needed for one test cycle is 60min. refer to table 10 for the detailed time arrangement.

Table 16 Sampling Phase Arrangement

Sampling point	Sampling time			remarks
S1	10min	25min	40min	The previous 10min is taken to wash the pipes.
S2	10min	25min	40min	The previous 10min is taken to wash the pipes.
S3	10min	25min	40min	The previous 10min is taken to wash the pipes.
S5	10min	25min	40min	The previous 10min is taken to wash the pipes.
S6	10min	25min	40min	The previous 10min is taken to wash the pipes.

B3. Sampling Methods

B3.1 Sampling Facility

A quantitative water sampler, plankton net and quantitative sampling bottles are to be used for sampling. Parameters are determined in field using salimeter, thermometer and the environmental factors are measured by a hygrothermograph.

B3.2 Sample Collection

B3.2.1 Collected samples for environmental parameters

Environmental parameters include Total Suspended Solids (TSS), Dissolved Organic Carbon (DOC), Particulate Organic Carbon (POC), pH value, Turbidity (NTU) and etc. They are detected in each sampling time interval. Collect 5L in each replicate, and the samples are to be stored in dark cool place and delivered to the lab as soon as possible. The Total Residual Oxidants (TRO) is to be collected using a brown bottle, the capacity of which is 100ml. And the bottle should be tightly closed with a stopper. Perform the field test according to the standard of *Water quality-Determination of free chlorine and total chlorine-Spectrophotometric method N, N-diethyl-1, 4-phenylenediamine HJ 586-2010*. The Dissolved Oxygen (DO) is to be collected using a brown bottle with rough cap, the capacity of which is 125ml. A quantitative liquid filling device is to be used to fill the sample with 1 ml manganese chloride solution and 1 ml alkaline potassium iodide solution

immediately after the sample is collected, Check the sample bottle cap for tightness (make sure no air bubbles in the containers after sealed), shake the bottle up and down for no less than 20 times with one finger pinning against the stopper and then take the sample to the lab for testing. The other parameters like the salinity and temperature are measured in field.

B3.2.2 The collection of organism samples (10-50 μm and $\geq 50 \mu\text{m}$)

B3.2.2.1 The collection of organism samples (10-50 μm)

Take samples at different sampling time, influent sample(control) 1L, treated water sample 10L, treated water 10L after a five day holding time, control water 10L after a five day holding time. The water samples with a size of 10L are taken by a water sampler to be filtered by the plankton net with a mesh size of 10 μm . The condensed water sample is placed in a specimen bottle with capacity of 60ml. Then it should be added 5 drops of staining solution for 15min, and then add some formalin to fix the sample. Add 2ml staining solution to the 1L of raw water sample for 15min and add formalin to fix the samples. After being settled for 24 hours, the supernatant is extracted, and the condensed sediment will be added to the specimen bottle with capacity of 60ml.

B3.2.2.2 The collection of organism samples ($\geq 50 \mu\text{m}$)

Water samples taken from the outlet will be filtered by a plankton net with a mesh size of 50 μm . Under the plankton net is a container with a capacity of 1m³. When the container is filled, stop filtering and the concentrated water sample will be collected into a specimen bottle with capacity of 60ml. After that add five drops of staining solution to the specimen bottle, staining for 30 minutes and then add the formalin for fixing. Take influent water samples (20L) at different time phases, and process them by the way as mentioned above.

B3.2.3 The collection of microorganism samples

A clean and sterilized glass container will be used when taking the microorganism sample. In order to avoid being polluted, the mouth of the sampling container must be sealed with alcohol cotton, and ignite it when sampling. If there is residual chlorine in the water sample, add sodium thiosulfate which is germ free to the glass container. The

volume of the water sample should be no less than 700ml.

B3.3 Sampling/Test System Failure Response and Remedy

Any sampling and testing interruptions or unexpected things occur during sampling should be given a due consideration and causes of them should be found. Report to the technical director in time and keep detailed record of the failure event. If it is resulting from instrument system failure, continue to test by applying another same instrument if necessary. Or else, repair immediately after report to the technical director.

Once the test is interrupted, cut the power supply in accordance with operation procedures.

There are circumstances when external accidents such as power failure, water failure and so on, happen which will affect the testing quality. Therefore, re-sampling and retest are needed when it returns to normal.

When it happens that the failure of instruments or apparatus causes the test to breakup. If there is a backup instrument, use it to replace the faulty one and continue the test. If there is only one instrument in the lab, and the failure of the instrument will affect the test quality, re-sampling until the instrument returns to normal. The instrument will be handled according to the Procedures for Instrument and Apparatus Management.

If something is wrong with the sample, stop the test and report to the technical director. Check the sample, find out the causes and make a suggestion. After being approved, decide how to solve the problem. Re-sampling and re-test are necessary.

Keep record of all abnormalities and interruptions occur in the handling process and fill in the sampling/test process and the result abnormalities handling record table. And report to the technical director.

B4. Sample Handling and Storage

B4.1 Sample Handling in Field

After samples of organisms with particle size between 10 μ m to 50 μ m are collected, add algae staining solution with concentration of 2ml/L to the sediment drum for 15

minutes. Then add formalin to fix the sample. After the samples are taken back to the lab, make the samples static for 24h and later use a device to absorb and filter the supernatant fluid of the phytoplankton. After 80% of supernatant fluid is filtered, make the sample stay static once again. After the sample is secondary absorbed to about 100ml, make it stay static for 24h for the third time, and then a constant volume 50ml is left for microscopic counting.

After sample of organisms with particle size greater than 50 μm , add five drops of formalin to fix the sample. Then the samples are taken back to the lab, settling down for 24h. After that filter part of the supernatant fluid and remove the samples to the 50ml cone centrifuge tube and keep making it settle down. Finally, filter the supernatant fluid and microscopic count the total number of the organisms.

If there is residual chlorine in the water sample, the samples are pretreated by adding certain sodium thiosulfate which is bacteria free in the glass container (0.1ml sodium thiosulfate per 120ml water sample, 15mg/L residual chlorine could be reduced by 10% sodium thiosulfate).

The dissolved oxygen (DO) is collected in a 125ml brown bottle with rough cap. A quantitative liquid filling device is used to fill the sample with 1ml manganese chloride solution and 1ml alkaline potassium iodide solution immediately after the sample is collected, Check the sample bottle screw caps for tightness(make sure no air bubbles in the containers after sealed), shake the bottle ups and downs for no less than 20 times with one figure pinning against the stopper and then take the sample to the lab for testing on the day arrived.

B4.2 Sample Storage, Transportation and Preservation Time

The samples of environmental parameters should be stored in dark. And the TSS, DOC, POC should be tested within 24h in room temperature. If the testing of DOC and POC can not be finished within 24h, add a small amount of HgCl_2 and it could be stored under -20°C for 7days. The remaining parameters such as TRO, pH, turbidity, and DO should be finished testing within 6h. The samples are placed in the dark after fixed in situ and transported to the lab; the test should be finished within 7days. The temperature should

be kept between 0~5 °C when the TRO and the bacteria samples are transported or stored, and the testing of them is demanded to finish within 4-6h.

After tested, the samples can be store for a long term being placed in fixing liquid and dark place. Add some formalin fixing liquid once three months to keep the samples from decay. Samples will be preserved for three months if there are no particular requirements by the clients. The bacteria samples should be tested right after transported to the lab for there is no way for long term storage. The environmental parameters samples are not suitable for long term storage after tested, if retesting is necessary, the holding time of the environmental parameters samples should be kept within the valid testing period.

B5. Test Analysis Methods

B5.1 Analysis of Organisms in Water Sample (10µm– 50 µm and ≥ 50µm)

B5.1.1 viable organisms greater than or equal to 10 micrometers and less than 50 micrometers in minimum dimension

Lightly absorb the supernatant fluid from the pretreated samples using a suction pipe with 10µm bolting silk. After settling down for a few times, the water sample is condensed to a 50ml thimble tube. Shake enough before sampling counting, absorb a certain amount of sample and then release it at the counting chamber covered with cover glass (make sure there are no bubbles remain) and then conduct the microscopic counting(GB17378.7-2007).

Optical microscopic counting (concentrated counting):

$$C = \frac{n \times V_1}{V_2 \times V_n}$$

Where:

C ——total amount of samples in per unit volume, unit:(cell/m³);

n ——number of samples, unit:(cells);

V_1 ——the volume of concentrated water sample, unit: ml;

V_2 ——volume of filtered water, unit:(m³);

V_n ——volume of sampling counting, unit:(ml).

Refer to appendix I -Test Methods and Organisms addition- B: Test method of organisms -1. Testing for organisms with a minimum dimension of 10~50μm for the detail.

B5.1.2 viable organisms greater than or equal to 50μm in minimum dimension

The samples being filtered and concentrated are identified and analyzed by total count method and counted by kind/species to calculate the organism number (number of organisms in per unit)(GB17378.7-2007):

$$\gamma_B = \frac{V_B}{V}$$

Where:

γ_B ——number of zooplanktons in per unit of volume;

V_B ——volume of sample, unit(ml);

V ——volume of filtered water, unit:(m³).

Refer to appendix I -Testing Methods and Organism Addition- B. Testing method for organisms -2. Testing for organisms with a minimum dimension greater than 50μm for introduction of the testing method for organisms with a minimum dimension greater than 50μm .

B5.2 Environmental Parameter Analysis

B5.2.1 pH value

Measured by pH meter (GB17378.4-2007).

Refer to appendix I -Test Methods and Organisms Addition- B.Test Methods of Environmental Parameters-1. pH value for detailed information about the determination of pH value.

B5.2.2 Turbidity (NTU)

Measured by turbidity meter (GB17378.4-2007).

Refer to appendix I -Test Methods and Organism Addition-B,Test Methods of Environmental Parameters----2. Turbidity for detailed information about the determination of turbidity.

B5.2.3 Salinity (PSU)

Determine the salinity in field using the USA kimcheon YSI 85-25 salinometer(GB17378.4-2007).

Refer to appendix I -Test Methods and Organism Addition-B,Test Methods of Environmental Parameters----3. Salinity for detailed information about the determination of salinity.

B5.2.4 water temperature (T)

Measured by reversing thermometer in filed (GB 17378.4-2007).

Refer to appendix I -Test Methods and Organism Addition-B,Test Methods of Environmental Parameters----4. Temperature for detailed information about the determination of temperature.

B5.2.5 Particulate Organic Carbon (POC)

Determination of particulate organic carbon (POC) by spectrophotometry. the carbon is wet oxidized by acidic dichromate, the decrease of the extinction value of the yellow dichromate solution may indicate the quantum of oxidized carbon “observation method for gulf ecosystem”. Calibrate the measured extinction value:

$$E = 1.1 \times E_f$$

Where:

E_f —difference of extinction value of sample and blank solution

Calculate the concentration of POC, unit: $\mu\text{g/L}$.

$$\text{POC} = \frac{E \times F \times v}{V}$$

Where:

V—volume of filtered seawater sample, L;

v—the volume of oxidation used in the C step;

factor F is calculated as follows:

$$F = \frac{120}{E_3}$$

Where:

E_3 —calibrated average extinction value of trivalent chromium at 440nm.value of F calculated is about 275.

Refer to appendix I -Test Methods and Organism Addition-B.Test Methods of Environmental Parameters----8. Particulate organic carbon for detailed information about the determination of particulate organic carbon,.

B5.2.6 (Total Residual Oxidant)

Determination of total residual oxidants in waters——N, N-diethyl-p-phenylenediamine (DPD) Spectrophotometry(HJ 586-2010).

Refer to appendix I -Test Methods and Organism Addition-B.Test Methods of Environmental Parameters----9. total residual oxidants for detailed information about the determination of total residual oxidants.

B5.2.7 Dissolved Oxygen (Dissolved Oxygen)

Determination of dissolved oxygen by iodimetry: A dissolved oxygen burette is applied to measure this water quality parameter.(GB17378.4-2007).

Refer to appendix I -Test Methods and Organism Addition-B.Test Methods of Environmental Parameters----5. Dissolved oxygen for detailed information about the determination of dissolved oxygen.

B5.2.8 Total Suspended Solids (TSS)

Determination of TSS -Gravimetric method: A certain volume of water sample passes 0.45μm membrane, dry and weigh the TSS left on the membrane, and calculate the concentration of the suspended solid in water (GB17378.4-2007).

$$\rho = \frac{W_1 - W_2 - \Delta W}{V}$$
$$\Delta W = \frac{1}{n} \times \sum^n (W_n - W_b)$$

Where:

ρ ——concentration of suspended solids, unit (mg/L);

W_1 ——weight of suspended solid plus membrane (W_2),unit (mg);

W_2 ——weight of water sample membrane, unit (mg);

V ——volume of water sample, unit (L);

ΔW ——calibration value of blank calibration membrane, unit (mg);

W_n ——weight of the blank calibration membrane after filtered, unit (mg);

n ——number of blank calibration membrane;

Refer to appendix I Part two -Test Methods and Organism Addition-B. Test Methods of Environmental Parameters----6. Total suspended solids. For detailed information about the determination of total suspended solids.

B5.2.9 Dissolved Organic Carbon (DOC)

The collected sample should be filtered by the Whatman GF/C fiber glass membrane which has preheated at 450°C for 5h and then tested by instrument (GB17378.4-2007). If the sample may not be analysis right away, add a small amount of mercury chloride to the sample and store it in the refrigerator.

Refer to appendix I -Test Methods and Organism Addition-B. Test Methods of Environmental Parameters----7. dissolved organic carbon. For detailed information about the determination of dissolved organic carbon.

B5.3 Bacteria Sample Analysis

B5.3.1 Determination of heterotrophic bacteria in water sample

Add 1mL of tween-80 solution to per 100mL of bacteria sample solution. Gradient dilution is made by high pressure sterilized seawater. Before the water sample is diluted, shake it with effort to make it mixed sufficiently. 10ml water sample is sucked by a sterilized suction tube and added to 90ml sterile dilution, getting a 10 times of dilution water sample. Shake it to make it well mixed. After that, based on the 10 times degree dilution, make the 100times, 1000times degree dilution in the same way as mentioned above and shake them to be well mixed. When the above mention diluting process is conducted, there is no need to change the sterilized suction tube. Take 0.1ml diluted water sample and spread it uniformly on the 2216E culture medium. Four dilution degrees of each water sample are needed to be prepared and replicate two plates for each dilution

degree. Put the plate into a constant temperature culture box (25°C) with its upside down for 7 days. Count the total number of the colonies with a stereomicroscope:

- (1) Do not count when large lawn appears on the plate.
- (2) Plate with number of colonies between 30 and 300 is selected; the average number of colonies multiplies the dilution degree (10 times, 100 times or 1000 times) equals to the number of the bacteria in water sample.
- (3) If there are two kinds of dilution degrees with average number of colonies are between 30 and 300, the ratio of the two numbers determines which one to choose. If the ratio is less than 2, the average of the two is chosen; if more than 2, the colony with less number is chosen.
- (4) If all the average values of all kinds of degrees of dilution are more than 300, the number of colonies is counted using the average number of colony in the largest degree of dilution (lowest concentration) multiplies the times of dilution.
- (5) If all the average values of all different degrees of dilutions are all less than 30, the number of colonies is counted using the smallest degree of dilution (highest concentration) multiplies the times of dilution.
- (6) If there are no colonies in all different degrees of dilution, and no inhibitor is tested, then report less than 1 multiplies the lowest diluted times.

Refer to appendix I -Test Methods and Organism Addition-II, test methods for organisms ----3.1. determination of heterotrophic bacteria. for introduction of the test method of heterotrophic bacteria.

B5.3.2 Determination of *Escherichia coli* in water sample

The samples should be tested by multi-tube fermentation method immediately after they are transported to the lab in cool conditions in accordance with the specification for marine monitoring—Part 7: Ecological survey for offshore pollution and biological monitoring GB17378.7-2007 and Standard examination methods for drinking water—indicator microbes GB/T5750.12-2006. Shake the water sample over 25 times at least before testing or diluting to make the water sample well mixed. 10ml water sample is sucked by a sterilized suction tube and added to 90ml sterile dilution, getting a 10 times of dilution water sample. Shake it to make it well mixed. After that, based on the 10 times

degree dilution, make the 100times, 1000times degree dilution in the same way as mentioned above and shake them to be well mixed. When the above mention diluting process is conducted, there is no need to change the sterilized suction tube. A 10ml of the original water sample is inoculated to the 10ml two fold lactose peptone culture medium; a 1ml water sample is inoculated to the 10ml unblended lactose peptone culture medium. Further more, take 1ml 10^{-1} , 10^{-2} , 10^{-3} diluted water sample to the unblended lactose peptone culture medium, five tubes of each degree of diluted water sample are inoculated. Put the inoculation tube into a culture box with a temperature of $36\pm 1^{\circ}\text{C}$ for 24 ± 2 hours. If no bubble or no acid is produced in the lactose peptone culture pipe, it indicates the *Escherichia coli* to be negative. If there is gas or acid produced, follow the steps bellows: inoculate the water sample in the fermentation tube to the eosin methylene blue agar plate, and put it in the $36\pm 1^{\circ}\text{C}$ culture box for 18h-24h. Observe the appearance of the colonies, and choose the kind of colony which gets the specific features(dark purple black, metallic luster; purple black, no or little metallic luster; light purple red, dark in the center) for Gram staining, microscopic test and verification test. If the water sample is tested to be Gram negative sporeless bacterium,inoculate to the lactose peptone culture liquid at the same time and put it into the culture box with a temperature of $36\pm 1^{\circ}\text{C}$ for 24 ± 2 h, if there are gas and acid produced, it is a proof of the existence of the *Escherichia coli*.

Test of the *Escherichia coli* in the tube which has fermentation phenomenon and gas or acid in it. A metal inoculation loop being sterilized by burning or the sterile cotton swab is used to inoculate the liquid in the tube mention above to the EC-MUG tube. Put the tube which has been inoculated into the culture box with a temperature of $44.5\pm 0.5^{\circ}\text{C}$ for 24 ± 2 h. The EC-MUG tube is radiated in the dark by a 6 w power UV lamp with a wavelength of 366nm, if blue fluorescent light is observed; it shows that there is *Escherichia coli* in the water sample. Count the number of the positive EC-MUG tubes, refer to the most probable number (MPN) table for the matched most probable number of the *Escherichia coli*, reported the result of the number of *Escherichia coli* in the unit of MPN/100ml.

B5.3.3 Determination of Intestinal Enterococci in water sample

The samples should be tested immediately after they are transported to the lab in cool conditions in accordance with the standards stipulated in water quality- detection and

enumeration of the intestinal *Enterococci* ISO7899-2-2000. Shake the water sample over 25 times at least before testing to make the water sample well mixed. connect the sterilized filter device to the Buchner flask, put the membrane at the bottom of the filter with a germfree tweezer, and certain amount of water sample is sucked to the filter, and be sucked and filtered by the vacuum pump. After all the water sample liquid passes through the membrane, clean the edges of the filter with 20ml to 30ml normal saline for twice at least. Then, turn off the vacuum pump and turn on the filter, and take the filtered membrane by a germfree tweezer onto the surface of the mEI agar culture medium- Slanetz and Bartley medium (membrane *Intestinal enterococci* culture medium). Ensure that no bubbles in the middle of the membrane and the medium. Put the plate upside down in temperature of $36^{\circ}\text{C}\pm 2^{\circ}\text{C}$ for $44\pm 4\text{h}$. After the culture time is over, all the colonies which is red, nut brown or pink, no matter in the middle or full over the plate are all typical. If there are typical colonies formed, transfer the membrane and the colonies using the germfree sweezer to the Bile Esculin Azide Agar plate which has been preheated to 44°C , culturing for 2h at $44^{\circ}\text{C}\pm 0.5^{\circ}\text{C}$, then observe the plate, if the color of the culture medium around the colonies is brownish black, it means the colonies are positive, these colonies is counted as *Intestinal enterococci*.(note: counting when the colonies are uneven distributed or bulge will affect the identification of the positive colonies. The color will diffuse to the colonies nearby). Count the membranes which are proved to be *Intestinal enterococci* colony, unit:(CFU)/100ml.

B5.3.4 Determination of *Vibrio cholerae* in water sample

The samples should be tested immediately after they are transported to the lab in cool conditions in accordance with the standards stipulated in the Diagnosis Standard for *Vibrio cholerae* WS289-2008. The samples collected are inoculated in the culture medium as soon as possible. Water sample of 450ml is inoculated in each 50ml, 10times concentrated alkaline peptone culture medium and it is put in a temperature of 37°C for 6-8h. And afterwards, take an inoculation loop culture from the bacteria film undersurface and streaking inoculated to the two kind of culture mediums ,the strong (Gentamycin agar, TCBS agar and No.4 Agar), and weak (alkaline nutrient agar) in 37°C for 18-24h. Cultivate

the strain identification of the typical colonies grown on the strong and the weak culture medium (Slide agglutination test, oxidase test and strain review).

The characteristics of *Vibrio cholerae* bacteria grown on different medium are various, the characteristics of colonies grow on the common culture mediums in 37°C for 18-24h are as follows:

(1)Alkaline nutrient agar: colorless, round, transparent or translucent, surface smooth, wet, flat or bulge slightly, edge neat, diameter of the colony about 2mm.

(2)Gentamycin agar and No.4 Agar: the characteristics of this kind of colonies are similar to those grow on the alkaline nutrient agar. But not so transparent, almost be translucent. And for that there is tellurite in the culture medium, the color of the center of the colony is usually grey or grey black and getting darker and darker as time goes.

(3)TCBS agar: yellow, glitter, surface smooth, wet, bulge slightly, edge neat.

If any questionable colonies found in the Serum agglutination reaction, send the colonies to the Shanghai Luwan District Disease Control Centre for subcontract testing.

B6. Quality Management Plan (QMP)

As the project test organization, the Ballast Water Detecting Lab of Shanghai Ocean University will carry out the project quality management in compliance with the Quality Management Plan QMP and takes part in the comparison of the testing results with those obtained by competent labs specialized in the same testing field, and participates in the proficiency testing program organized by authorized organization according to the lab file which is called the Procedures for Testing Result Quality Control.

Retest of the samples in the retention time, retest the same sample by the same method or different method, and retest the same sample using the same instrument or different instrument to assure the quality of the test result. Keep the sensitivity, accuracy, deviation allowance range, precision of the parameters; ensure the reliability and integrity of the data.

Enhance the quality awareness of the test personnel; make a clear division of quality responsibilities. The test undertaking organization should be supervised by the entrusted

organization and the technical supervision organization. The test undertaking organization should take the quality control procedures in the test process into the quality operation system and make the quality plan to comply the quality system and requirements of the testing project.

B6.1 Quality Management of Field Sampling

Assure the quality of field sampling and analysis. Prepare the procedures for conducting the sampling and avoid the samples being polluted. Keep away from the interference of the instruments. Select sampling facilities, sample bottles appropriate for the testing items. Take antipollution measures for accessories in testing place; minimize the influence of the interface concentration. The pretreatment of samples should be completed in situ right after the samples are collected, and then add some stabilizer and store in low temperature. Items which are susceptible for microbes' activities or change fast with time should be finished testing within the stipulated time.

Indicators like salinity, water temperature, conductivity should be measured in field. During the field test, turn on the instrument and leave it to be warmed-up until the instrument reading and the flow of the ballast water management system become stabilized, and then wash the sample bottle for two times with a little of water sample, afterwards, fill the bottle up with sample, the probe of instrument in the sample bottle, get the reading after the instrument is stabilized. When collecting the suspended solid water sample, wash the sample bottle for two times with a little of water sample, and then fill the sample bottle to the fully slowly. Ensure the water samples are stored in cool and shady place and transported to the lab. Ensure the water sample be filtered within 24h. The DOC and POC samples collected simultaneous are divided in the lab; try to use the ground glass sample bottle to collect the samples in order to avoid the absorption of C of plastic products. Before use, all the glass containers should be immersed in the Sulfuric acid and potassium dichromate lotion for 24-28h; then be rinsed with tap water and washed again with de-carbonized water, and the de-carbonized water should be prepared in advance. When collection samples in situ, wash the sample bottle with a little of water sample first and then collect the samples and refrigerated transport the sample to the lab.

Collect of 10-50 μm viable organisms sample by method of sediment and concentration counting of certain volume of water sample. There is no need to rinse the sample bottle when collect the samples, collect certain amount of well mixed water samples. Water sample of viable organisms greater than 50 μm is prepared by collecting the organisms filtered by a 50 μm screen organism net and count the total number. Ensure the organism net is clean and tried before collecting. Viable organism staining agent should be prepared temporarily (to avoid the failure of staining agent as time goes) before sampling. Add quantitative staining agent to the collected viable organism sample for enough time and then add Formalin to fix the sample. Right after the viable organism samples are collected, transport them to the lab (protect them from light and vibration) and analysis as soon as possible.

The microbe sampling and test personnel should be trained and be equipped with basic knowledge of microbes. When conduct the microbe testing, all the containers should be sterilized. Prepare a set of trip blank and field blank during each sampling process. One reagent blank should be prepared in each batch of samples or ten samples. Keep record of all the original data of the initial dilution water samples for review. Each dilution degree of water sample should replicate.

B6.2 Quality Management of Lab Sample Analysis

B6.2.1 Quality Management of chemical reagents

Chemical reagents used in lab sample analysis should be prepared to solutions in accordance with prescribed conditions. The solutions should be stored in right conditions and used within the prescribed period. The self-prepared solutions are allowed to use unless they are calibrated to be qualified with the guarantee value of national standard solution. The blank value of reagent should be in the same level with the analysis detection limits. If the value is too far over the detection limits, the causes need to be found. And main agents are purified which have great agent blank value or change the reagents (use a new batch number of agents or agents produced by other manufacturers). And all the reagents should be checked before use. In the cases when the blank value is hard to be

lowered, add appropriate amount of reagent. During analyzing, parallel test the analysis blank and monitor the variation of the blank value.

B6.2.2 Quality management of containers

Make a clear understanding of requirements for the materials used in containers, select the right material. The characteristics of container material should have the least pollution to the water sample and be easy to clean. And it should be inertia to the chemical activity and biological activity to protect the water sample from reacting with the container to the maximum extent. The capacity of dealing with temperature fluctuation, resistance to rupture, sealing property, capacity of reopening, volume, shape, mass and possibility for reuse of the sample storage containers should be taken consideration when selecting the containers. For most samples which include inorganic compositions, containers which are made of polyethylene, polytetrafluoroethylene or eater polymer are chosen to use; for the storage of samples for determining and analyzing the conductivity and PH in water, containers which are made of high density polyethylene are used; for the storage of organic chemical and organism samples, glass containers are used. The containers should be cleaned in the right way; the compositions of the detergent should not include the substance to be tested. The new container should be cleaned thoroughly; the substances to be tested determine which detergent to choose. For general use, taps water and detergent are used to clean dust and packaging matter, then immersed in the chromic acid and sulfuric acid detergent, and at last rinsed with stilled water. For those used containers, there are usually grease, heavy metal and residents in the bottom and wall of the container, there once they are reused, and they must be cleaned before being used. For those glass containers with stoppers, the ground part is often with digestions and absorptions. Polyethylene is susceptible to absorb oil or grease, heavy metal, sediments and organisms and it is hard to clean. So, much attention should be paid when cleaning the containers made of polyethylene. Before the container made of polyethylene is used, clean with 1 mol/L hydrochloric acid solution and immersed in the (1+3) nitric acid solution for a long time. Before the sample bottle used for storage and environmental parameters analysis is used, clean it with nitric acid solution, and then rinse with stilled water to remove the heavy metal and chromate residual. If the organic composition to be determined is tested

after extraction, the glass bottle may be cleaned with extraction detergent.

B6.2.3 Quality management for instrument

The analysis instrument for testing should be in compliance with the stipulations of the Specification for Ocean Monitor GB/T17378. Instruments are checked and calibrated by specified personnel in regular times. The instruments should be cleaned with still water after being used and immersed in the protection liquid to avoid the residual of samples and corrosion of the instruments. Or maintain the instruments according to the instrument operation manual to keep off of measurement error next time, and conduct the instrument interval check as necessary.

B6.2.4 Quality management for environmental parameters

After the DOC and POC samples are collected, use fiber glass membrane with 47mm diameter and 0.45 μ m diameter and the standard micropore filter to treat the samples. The membrane must be burned in the 450-500 $^{\circ}$ C muffle for 24h wrapped in aluminium foil to remove the oxidizing substances (the burning temperature should not exceed 500 $^{\circ}$ C, or else, the filtration characterization of the membrane will change). When the TSS is filtered, clip the membrane with a stainless steel tweezer for fear of pollution. Prevent the seawater from flowing backward and then damage the vacuum pump. And drain out the wastewater in time. Keep the ambient tidy when drying the sample.

Analyze by drawing the standard curve for determination of DOC. Firstly, prepare new chemicals for drawing the standard curve. To assure the quality of the value determined, only if the accuracy of the standard curve reaches to 98% or above, the standard curve is valid.

B6.2.5 Quality management for lab test methods

The lab can undertake the test task on condition that it is accredited the Metrology certificate. Test method is selected mainly based on the precision, accuracy and detection limits of method, to give due consideration of factors such as cost, instrument condition and test cycles and the skill level of personnel. The test methods used should be verified by standard novelty search.

B7. Performance Test, Checkup and Maintenance of Test Instruments

The instrument manager is responsible for compiling the Check List of the Instruments, and establish the file of instruments and identify the instruments with related labels. The operator of instruments should be an authorized staff or one with vocational test staff certificate. All operators should be approved by the lab to carry out the operation. Use of equipment should be strictly in accordance with operating procedures. The staff is asked to operate the instrument as trained to be in order not to get the invalid test result. The user of the instruments should check the status and environment condition of the instrument (including whether it is in valid period, need maintenance or not, if stabilized or not) before and after use. And fill the Use Record of the Instrument.

If there is abnormal phenomenon (overloading, wrong operation, questionable result displayed) for the equipment, the user should stop the operation and stick a red mark on it. Separate the abnormal instrument to avoid misuse. If the instrument falls out the direct control of the lab for example: removed to other places, sent for repair or calibration, after the instrument is back, the instrument attendant should check the functions and the calibration status of the instrument and recovered to use until the results displayed are satisfying.

The instrument manager takes charge of checking the instruments to prevent the instruments from damaging and losing. Make an inventory of the instruments annually. If there is damage or lose of instrument, repair or handle in accordance with the Control Procedures for Nonconsistent Test Work

A specified worker is appointed for the maintenance of the instrument in use. Power on once per month at least (1-2h) to check if the instrument is normal and keep record. The instrument manager is responsible for organizing the instrument user to make the routine maintenance plan, and to form the Routine Maintenance Table of Instruments. The instrument user makes the maintenance of the instrument to comply with the items and periodic times in the table and keep the record meanwhile.

B8. Calibration and Frequency of Instruments

Instruments like the spectrophotometer, electronic balance, pH meter, turbidimeter need to be verified and calibrated by legal metrology verification service agency. The instruments are delivered by the lab synthesizer according to the Quantity Traceability Procedures, aiming to get the qualified certificate. The instrument attendant performs the periodic calibration of the instrument. If the correction factors are obtained after instrument calibration, the instrument attendant is responsible for updating of all the backups and the correcting of related data. The frequency for calibration is once per 12 months, once per 6 months for special instrument.

B9. Data Collection Requirements

The project technical director summarizes the results obtained both from filed work and lab test for land based test and organizes the data acquisition and statistics. Prior to data statistics, the test personnel should check the test data first. Check if the original data is integrated and if it meets the requirement, if the calculation and conversion of data is right. Mutual correction is preferred by test personnel after the test data is checked by the test personnel. The reviewer should carry out the review in conformance with the standards, procedures, norms and enforcement rules, and if calculation is required, the calculation formulas and the calculation process should be checked. Check whether the calculations, the rounding off and the conversion are right. The reviewer should review the original data thoroughly at the time of checking the test reports for the reliability and the matching of the data. The data verified to be right is collected and summarized by the technical director.

B10. Data Management

The sampling data and data determined in field should all be record in waterproof table or to create the electronic document right after the samples are taken in field. The management of the electronic data is in accordance with the Procedures for Computer Management.

B10.1 Data Records

To make the record meet the standard and ensure that sufficient information is collected, the lab adopts the uniformed and approved form of record table. The sampling records should be prepared with pen or ball-pen. The handwriting should be clear. And the record should be verified and integrated. There should be date of record, signature of recorded person and the record number on the record, and the technical record should include the signatures of test personnel and reviewers. The technical record should include technical parameters. All the technical parameters, data, observation results and calculations should be kept being recorded in time, with no replenish.

If the sampling records need to be modified, two lines should be written on the original records, which should be made out. Then the modified records should be written on the blank on the top right of the original records with the mender's stamper or signature or abbreviative signature. All the records should be collected, filed and preserved.

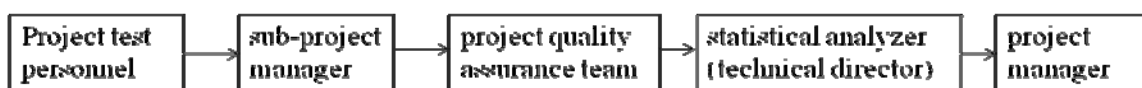
B10.2 Data Confirmation

The test personnel conduct the test in accordance with the requirements and standards related. The quality supervisor takes necessary actions to oversight the test. After the test is finished, the technical director need to sign for confirmation.

B10.3 Data Conversion

The quantity of the plankton is counted and converted to the uniform unit required by the project by statistical analysis. Thereof, organism samples with particle sizes between 10 μ m and 50 μ m are converted into cell/ml; particle sizes greater than 50 μ m are converted to cell/m³; the bacteria samples are converted to cfu/100ml.

B10.4 Data Delivery



B10.5 Data Analysis

The original data record is collected by related test personnel, and the test personnel will calculate the final test results according to the conversion method of the parameters and the corresponding curve. The calculation process of data is included in the original record. Take the assessment for the uncertainty occurs in the testing process based on the stipulations in the standards for testing all parameter and determine the significant digits for values. At last, the technical director analyzes the removal rate of organisms with different particulate diameters at each steps of ballast water management, and calculates the removal effect. The rounding off method of specific values is as follows:

1) Refer to *The Rule of Data Revising* (GB8170-87) for rounding off the values

The rule for rounding off of the numerical values is a round 5(the digit of the tested valid number is determined): when the rounding off number of the measured value is less or equal to 4, then rounding down; if the rounding off number in the measured value is more than or equal to 6, then rounding up; if the rounding off number in the measured value is equal to 5, rounding up if the mantissa rounded up number is even number, and rounding down if the mantissa rounded up number is odd number. The measured values are rounding off by this way.

In calculating and reading of data, the digits of data might be more than prescribed, for example, the digits of data calculated in calculator may be 7, and when weighed on the analytical balance, only 5 digits of data is obtained, so it is necessary to rounding off the redundant digits. The process for cutting the redundant digits or digit is called the rounding off process, and it is in accordance with the rules of Four Rounding Down and Five Rounding up.

2) Data calculation rules

The data calculation rules are determined by the law of error transmission.

Plus- minus method: transmission of the absolute errors of the measured values. The absolute error of the max absolute error of measured values determines the uncertainty of the analysis result. Therefore, the retention of the significant digit of the summed value of several measured values should base on the number which has the least digits after the

decimal point.

Multiply-division method: transmission of the relative errors of the measured values. The relative error of the result should be in accommodation with the value with the max relative error. Therefore, rounding off of the values should be in accordance with the least significant digits.

Scale values of the volumetric containers used for Titrimetric analysis (burette, volumetric flask, pipette) are all with four significant digits. So the number of significant digits of the test data result is four.

3) Formula for calculating removal rate:

$$\text{removal rate} = \frac{\text{density before treated} - \text{density after treated}}{\text{density before treated}} \times 100\%$$

B10.6 Data Storage and Retrieval

All the test data record should be kept by the data administrator. The preservation time of the copies of the original test record, test reports is five years and the data administrator takes charge of the safe custody of the files and records. The records should not be let out or loaned to people unrelated and the customer's business secret should be kept.

The internal staff should go through procedures for loan or copy of the documents, and he or she should fill in the registration table. For external staff who wants to loan or retrieve the records, he or she should be approved by technical director, after the technical director give approval, he or she can go through the loan procedures and fill in the registration table. Read on site, no taking away. The user or keeper of the records should comply with the procedures for keeping the secret and proprietary of custom, do not copy without permission and forbid revealing.

C. Evaluation and Supervision

C1. Evaluation/supervision and Response Actions

Project supervisor of the land-based ballast water management system test project is responsible for conducting a continuous improvement actions based on the quality policy, quality objectives, approved result, data analysis, data correction and preventive measures and management review of the lab: analyzing and assessing the status in quo, looking for and finding the aspects needed to be improved(looking for the improvement opportunity); Ensuring improvement aims; establishing improvement scheme and reviewing the scheme, then selecting the best one; Implementing the responsibilities and related resources, and putting forward the improvement scheme; Monitoring and measuring the implementation situation to make sure whether the it is effectively implemented; formally taking the effective measures; the corrective and preventive actions should be taken into the plan and the management of daily improvement activities.

To determine the causes of discrepancies and look for the improvement chance by way of internal approval, management review, custom feedback, ability verification or other way of data analysis of quality control result. If preventive measures are taken, supervise and monitor the implementation of them, to minimize the possibility of nonconformities and look for improvement chance. Conduct the assessment in accordance with the lab' improvement control procedures, correction measures procedures, preventive measures procedures, test result quality control procedures and the management review control procedures and take the emergency response measures.

C2. Test Report

The technical director of the test organization submits the test reports, and the quality manager of the test organization submits the uncertainty report to the quality management team of land-based test for ballast water management system. The supervisor of the test organization briefly summarizes the results of related parameters and proposes a new

project quality assurance plan to the supervisor of the entrusted organization for summary and renewal.

The testing report of each item and the test results should be precise, clear, objective and conducted in compliance with the test methodology.

Each test reports should include at least information as follows at least: test designing, identification of the methodology, status description of the tested material and cleared label, the acceptance date and the test date, the test result, the test report approver or equivalent mark; if the test result need to be explained, there should be announcement about the test method deviations and evaluation uncertainty included in the test report. In the cases when the testing results provided by a subcontract party are included in the testing report, those results should be marked clearly. The subcontract party should report the results in the way of paper edition or electronic edition.

D. Validity and Usability of Data

D1. Review, Verification and Validation of Data

Check and review all the data from field determination and lab test and verify the integrity, continuity, validity of the data, and check whether the items meet the requirements. Conduct the comparison between the data and the quality objectives set in A7. When the data results are in consistent with the quality control data of ballast water management system and the data quality achieves the objectives of this project, then the ballast water management system is acceptable.

D2. Verification and Validation Methods

The review, verification and validation of data should be performed to ensure the data meets the criteria. Verify and compare the data with the planned data objectives described in document A7. The verification and validation methods include self assessment, taking part in the reconciliation activities with other labs organized by authorized parties and the ability verification plan. The authorized signatory verifies the quality control data by

statistical technology annually and makes the verification reports, then input the management review. If the quality control results are not satisfying or unstable, look for the causes for problems and take actions in accordance with the lab's Corrective Measures Procedures, Discrepancy Test Work Control Procedures, and Preventive Measures Procedures. The data validation includes all the task plans of the ballast water management system test except the data verification confirmation, including the quality control result assessment for determination of field sampling data , assessment for determination of lab parameters, discrepancy analysis of sample storage and pretreated, the sample test limitation time range verification, the traceability of methodology for test reagents and test standards, verification of the analysis sensitivity in conformance with QAPP, deviation analysis of sampling and analysis with requirements of QAPP, the verification of calculated results, to ensure that QAPP includes relevant information on all the parameters and samples.

D3. Reconciliation with Test Data Result Objectives

Data generated in this project is analyzed and reconciliated with the data quality and project requirements in accordance with the guidelines for approval of ballast water management systems G8 and discharge requirements of ballast water D-2 regulation. The data meet the requirements of the project and the D-2 standard, and achieve the treatment effectiveness of ballast water management systems and the data related documents will be applied to the authorized organization as appropriate.

E: Appendices

Appendix I. Test methods and organism addition

AppendixII. Qualification certificate of the test organization